THE CHARACTERIZATION OF VOLATILE MATTER CONTENT IN CHARCOAL AND ITS IMPLICATION FOR SOIL FERTILITY

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By Tai McClellan

Thesis Committee:

Jonathan Deenik, Chairperson

Nguyen Hue

Qing Li

Goro Uehara

Student:	Tai McClellan
Student ID#:	16454795
Degree	MS
Field:	Tropical Plant and Soil Sciences
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We certify that we have read this Thesis and that, in our opinion, it is satisfactory in scope and quality as a Thesis for the degree of Master of Science in Tropical Plant and Soil Sciences.

Thesis Committee:

Names	Signatures
Jonathan Deenik, Chairperson	
Nguyen Hue	
Qing Li	
Goro Uehara	

Abstract

We conducted laboratory studies to characterize the volatile matter (VM) content of charcoals and related differences in charcoal VM content to key aspects of soil fertility. In the first study, we characterized charcoals with varying VM contents and feedstocks using fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR), and determined the chemical composition of the acetone-extractable fraction of charcoals using gas chromatography-mass spectrometry (GC-MS) and total phenol content (Prussian Blue). We found the VM content of charcoal primarily consisted of alkyl carbons, oxygen-substituted carbons, and phenolic compounds. However, the GC-MS data indicated that charcoals can differ vastly in their extractable fraction, depending upon both VM content and feedstock. In a second set of experiments, we examined the effect of VM content and feedstock on soil microbial activity, nitrogen, and soluble carbon. High VM corncob charcoals significantly enhanced microbial activity, coupled with net reduction in nitrogen and soluble carbon. For a given feedstock, the extent of this effect was dependent upon VM content. The effect on microbial dynamics was apparently related to the acetone-extractable fraction of charcoal. The removal of the fraction from charcoal decreased its effect on microbial activity, while the addition to fungal inoculum increased its growth and activity. In our third experiment, we incubated charcoals and charcoal/soil mixtures and developed charge fingerprints. We found that high VM charcoals developed net negative charge upon aging, whereas the low VM charcoal did not. However, the addition of high VM charcoals did not result in substantial improvement in soil CEC. While we showed that VM content and feedstock influence charcoal behavior in soil, further research is needed to better characterize VM and its variation with feedstock in order to understand its effect on chemical composition and charge properties.

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1.1 Introduction

The sequestration of carbon and the enhancement of soil quality are among the most imminent challenges imposed upon the world's arable lands. While the former concern aims to combat global warming by offsetting anthropogenic fossil fuel emissions through the capture and storage of carbon in soils (Marris, 2006), the latter involves the implementation of practices that provide adequate food, fiber, and fuel without hindering soil health and function (Karlen, 1997). Different strategies have been proposed for offsetting greenhouse gas emission by carbon sequestration, including reforestation efforts, the pumping of carbon dioxide into deep geologic layers, and the enhancement of soil carbon stocks (Lehmann et al., 2006). The soil system is a promising means of storage carbon since it acts as a carbon "sink" for approximately 80% of terrestrial carbon. However, soils generally exhibit a low potential for carbon accumulation and are particularly influenced by land use, such as agricultural activity. Lehmann et al. (2006) propose that the best approach to terrestrial carbon sequestration is by the additions of biomass-derived bio-char (or charcoal/black carbon), which provides the opportunity for long term carbon storage and simultaneously conditions soil for improved soil fertility and increased crop production.

Charcoals consist of a range of materials that can differ widely, particularly in their chemical structure and composition. Such differences among charcoal types largely determine the effect of charcoal on soil properties. Through a series of greenhouse studies, we showed that one charcoal can elicit a very different plant response than another (Deenik et al., 2010). In our first greenhouse study, we examined the effect of various rates of macadamia nutshell charcoal in a volcanic soil on lettuce growth. We determined that the charcoal negatively

impacted the growth of lettuce at the highest rates of addition at high rates. We performed two additional greenhouse studies, with corn as our test crop in a highly weathered soil, and obtained the same results. A prior group of researchers also found that charcoal can have a detrimental effect on plant growth (Gundale and De Luca, 2007). However, these researchers suggested that the degree of carbonization is a determinant. Particularly, low-temperature, or partially carbonized, charcoals produced a negative effect on plant growth; whereas, higher temperature charcoals did not.

Differences in the degree of biomass carbonization can result during pyrolysis, and even within one batch of charcoal (Varhegyi et al., 1998). Volatile matter (VM) content of charcoal is an easily measured property (Antal et al., 2000) that is inversely related to carbonization (Antal and Gronli, 2003; Meszaros et al., 2007). Typically, a charcoal with a low VM content (less than 10% VM content) is more closely related to "pure" charcoal, or graphite. Such charcoal is suitable for metallurgic use charcoal. In contrast, a high VM (20-35%) charcoal is less carbonized and more suitable for use as a slow-burning barbeque charcoal. Subsequent to our initial greenhouse studies, we determined that the macadamia nutshell charcoal that we had used in our early greenhouse experiments was partially carbonized and contained high volatile matter (VM) content. Upon the discovery that charcoals can differ significantly in their VM content, we tested its effect on plant growth. We conducted a fourth study which compared the impact of a low VM and a high VM macadamia nutshell charcoal on corn growth in a highly weathered subsoil. We found that the high VM charcoal reduced corn yield, while the low VM charcoal did not. In fact, the growth of corn appeared to be enhanced by the combination of low VM charcoal and fertilization. From this investigation, we concluded that VM content in charcoal

was an important charcoal property that was responsible for the differential impacts on plant growth.

Being a rough estimate and comprising an array of compounds, VM content has not been extensively characterized. This thesis research examines VM content in charcoal and its effects on soil processes. As an easily measured property, we aim to determine whether the VM content can help to predict charcoal behavior in soil and its effect on plant growth. However, this is not possible without first characterizing the chemical structure and composition of charcoal and then relating this information with its behavior in soil. Particularly, further information is needed to identify the effect of VM content on changes in soil fertility (e.g. CEC and nitrogen dynamics).

1.2 Literature Review

1.2.1 Terra Preta do Indio

In an extensive review by Glaser et al. (2002), the authors show that the acknowledgment of charcoal as a soil amendment has garnered international attention upon the discovery that charcoal is at least in part responsible for the prolonged fertility of the Terra Preta do Indio (Terra Preta) soils in the Brazilian Amazon basin. Scientists now recognize that these soils are atypical due to their relatively high soil organic matter and nutrient levels, such as nitrogen, phosphorus, and calcium (Glaser et al., 2000). Terra Preta were created by the activities of ancient civilizations. Archaeologists propose that pre-Columbian Indios produced charcoal in smoldering pits under reducing conditions, of which was added to soils along with bone and green waste materials. These anthropogenic black soils occur in plots averaging 20

hectare and date more than 2,000 years old. In comparison to the typically, infertile adjacent Oxisols, Terra Preta sustain greater yields (Lehmann et al., 2002) and shorter fallow periods (German and Cravo, 1999, in Glaser et al., 2002). Furthermore, estimates suggest that the soils sequester almost 3.5 times the amount of carbon as compared to the less fertile, adjacent lands (Glaser, 1999).

Scientists now confirm that the Amazonian black soils contain 70-fold more pyrogenic carbon than surrounding soils (Glaser et al., 2001). In a study of a Terra Preta soil by Lehmann et al. (2005), the researchers estimated that charcoal was deposited 6,000 years BP, and the soil was largely amorphous dominated by aromatic carbon in addition to carboxylic groups. Black carbon appeared to consist of a highly aromatic core, which became increasingly oxidized outward from the center of the core. A second, non-continuous region which surrounded the core contained greater carboxylic and phenolic carbon. This outer region was presumably developed by microbial and abiotic oxidation in the soil, and showed strong adsorption of dissolved organic matter. The presence of such oxidized functional groups may be related to high cation exchange capacity and surface charge measured in soils containing historic charcoals (Cheng et al., 2008). In another study, Kim et al. (2007) showed that Terra Preta soils differed in their bacterial diversity relative to a pristine Brazilian forest. While they supported a similar community composition, the black soils maintained approximately 25% greater species richness. Thus, scientists have found that Terra Preta soils are unique in its amounts of aromatic carbon, oxidized particle surfaces, and richness in microbial species.

1.2.2 Terra Preta Novo

Proponents of using charcoal for large-scale farming and carbon sequestration are part of the Terra Preta Novo, which seeks to use the Terra Preta phenomenon as a model for modern sustainable agriculture (Marris, 2006). There are several findings which attest to the agronomic value of charcoal (Glaser et al., 2002). First, the application of charcoal can have a liming effect, which results in an increase in soil solution pH and decrease in aluminum saturation (Mbagwu and Piccolo, 1997). Secondly, charcoal appears to enhance the nutrient retention of highly weathered soils, due to its adsorbent character (Tyron, 1949; Glaser, 1999). In particular, charcoal has been shown to undergo oxidation and form carboxylic groups along its aromatic backbone which can form organo-mineral complexes and increase cation exchange capacity. The adsorption of metal ions and dissolved organic matter reduces the losses of nutrients due to leaching (Glaser et al., 2000). Thirdly, charcoal improves physical properties of the soil, including enhanced water retention and structural stability (Piccolo and Mbagwu, 1990). Fourthly, charcoal can be a direct source of plant available nutrients, including free bases of calcium, potassium, magnesium, and available phosphorus (Lehmann et al., 2002).

1.2.3 Charcoal Vision

The Charcoal Vision is a comprehensive plan which advocates the utilization of charcoal in terrestrial systems for the dual purpose of improved carbon sequestration and soil fertility. Its mission is to implement integrated agricultural biomass-bioenergy systems which improve both soil quality and productivity (Laird, 2008). Cellulosic biofuel plants can serve as potential

sources of energy via the production of ethanol. Pyrolysis is an energy efficient method of processing biomass which can thermally transform approximately 60% of the total biomass into bio-oil. However, a shortcoming of biofuel plant production is the requirement of adequate mineral nutrition for growth, which is removed from the soil upon harvest. In regard to this limitation, a second benefit of pyrolysis is that it yields charcoal as a by-product. During pyrolysis, approximately 20% of the total mass is converted to charcoal, which retains some nutrients and can thereby be returned to the soil as a largely stable carbon source. Laird (2008) estimates that not only is there potential to displace 1.91 billion barrels of fossil fuel per year (~25% of United States annual consumption), but 139 Tg carbon per year could be sequestered as relatively "fixed carbon."

Corporations have expressed interest in the development of the Charcoal Vision. Eprida, Inc. and the National Renewable Energy Laboratory have developed a profitable way of producing hydrogen from the gases and liquids formed during pyrolysis (Day et al., 2005). A portion of the hydrogen can be utilized to produce ammonium and subsequently combined with the charcoal by-products. The resultant is a slow-release, nitrogen-impregnated, carbon-sequestrating fertilizer, which accomplishes both goals of carbon sequestration and enhanced soil fertility.

1.2.4 Flash Carbonization™ charcoal

Opportunity exists in Hawaii to utilize charcoal resources in agricultural sciences. Researchers at the Hawaii Natural Energy Institute have developed a fast and efficient method for biomass carbonization. This process, known as Flash Carbonization™, produces charcoal from a packed bed of biomass via the ignition of a flash fire at high pressure (Antal et al., 2003).

Biomass can be converted to charcoal in only 20 to 30 minutes, due to the rapid spreading of flames at such elevated pressures (1MPa) and temperatures reaching approximately 400°C. Whereas traditional methods of producing charcoal generally yield less than 80% of fixed-carbon and require several hours to days, Flash Carbonization™ can yield up to 100% of the theoretical limit for fixed-carbon in only minutes (Antal et al., 2003). Furthermore, this process can utilize a variety of feedstocks, including wood, agricultural by-products, green and sewage wastes, and synthetic materials (Antal et al., 2003; Yoshida and Antal, 2009). The Hawaii Natural Energy Institute is currently testing a commercial Demonstration Reactor on the campus of the University of Hawaii at Manoa, which has successfully carbonized corncobs in less than 30 minutes at a commercial scale (Antal et al., 2003). Though an efficient technique, studies have shown that charcoals can vary significant in their VM content, even within one batch of charcoal (Varhegyi et al., 1998). Therefore, VM content is routinely measured as part of their proximate analyses.

1.2.5 Chemical properties of charcoal

Like any soil amendment, we can optimize the value of charcoal by first understanding its chemical properties. The study of pyrogenic materials is complicated by the fact that it represents a vast array of compounds. The continuum ranges from partially charred plant residues, charcoal, soot, and graphite. During the combustion process, solid residues form char, while soot can be produced by the recondensation of volatiles (Knicker, 2007). The defining characteristic of charcoal is the presence of condensed polyaromatic structures (Schmidt and Noack, 2000). Soils contain a continuum of pyrogenic carbon, depending upon the extent of thermal alteration. Charcoal that has been increasingly thermally altered (high temperature charcoal) has fewer and fewer functional groups which contain oxygen and hydrogen, in

comparison to less thermally altered charcoal (low temperature charcoal). As a result, high temperature charcoal is typically more carbonized than low temperature charred residues. As thermal alteration increases, small cross-linked aromatic structures yield bigger graphene sheets which are characterized by a disordered packing of these stacks. Soot particles also condense and envelop the graphene stacks. In comparison, graphite is pure carbon and characterized by highly ordered, parallel graphene sheets. Though produced during combustion, polycyclic aromatic hydrocarbons with 2-7 fused ring structures are not considered to be part of the pyrogenic carbon continuum (Preston and Schmidt, 2006).

The degree of carbonization is dependent upon the temperatures at which major chemical and structural changes occur in the biomass. Plant residues undergo a series of transformations during the charring process as the peak temperature continually increases. Below 220°C, the formation of water constitutes most of the weight loss from cellulose. While oligosaccharides are preserved, unsaturated single carbon-to-carbon bonds and carbonyl groups form due to the loss of water. However, up to 250°C, carbon dioxide and carbon monoxide are also evolved. Above 250 °C, char is characterized by the appearance of phenol and furan structures. As heating temperature exceeds 290°C, charcoal becomes dominated by alkyl furans, benzenoid aromatics, and condensed aromatics (Antal and Gronli, 2003). Knicker (2007) reported that phenols, furans, and aromatic hydrocarbons increased in abundance as temperatures rose from 250 to 310°C. An increase in temperature from 300 to 500°C results in an increase in the amount of fixed-carbon as the relative oxygen content decreases. Alkyl-aromatic groups and oxygen-containing functional groups, including hydroxyl, carboxyl, carbonyl, ether, and lactone structures can populate the surfaces of char depending upon its formation temperature. However, at 650°C, hydroxyl, C=O, and aliphatic carbon-to-hydrogen

groups are mostly absent. Most aromatic carbon-to-hydrogen groups are degraded by 750°C, and by 950°C, charcoal resembles graphite. Porosity, surface area, and adsorption properties also increase with increasing formation temperature (Antal and Gronli, 2003).

The volatile matter (VM) content of the charcoal is a chemical property that is affected by the degree of carbonization. For instance, less carbonized biomass has a relatively higher VM than more carbonized materials. The VM content is measured weight loss when charcoal is heated to 950°C for 6 minutes (Antal et al., 2003), and generally ranges from 10 to 40%. At 950°C, any remaining volatile matter of charcoal is evolved and almost pure carbon remains. However, below 600°C, light organic compounds and gases are lost. In contrast, the loss of volatile matter predominately consists of water, carbon dioxide, carbon monoxide, hydrogen and methane evolution at peak temperatures exceeding 600°C (Antal and Gronli, 2003). Thus, low temperature charcoals (<300°) derived from cellulose can be characterized by yields containing furans, pyranose, anhydrosugars, whereas polycyclic aromatic hydrocarbons become abundant at temperature ranging from 300 to 600°C. Lignin components volatilize mostly substituted methoxyphenols between 500 and 600°C (Knicker, 2007).

In comparison to plant residues, charcoal is largely resistant to decomposition, but it is ultimately subjected to slow degradation in the soil environment. Czimczik et al. (2003) found that charred materials collected from the Boreal forest after a wildfire were characterized by small clusters of aromatic region and a high degree of functionality. Enhanced functionality can typically make these charred particles more susceptible to degradation. As charcoal ages in soil, the particles become progressively more chemically oxidized and contain a greater proportion of carboxylic and phenolic functional groups (Lehmann et al., 2005). Further oxidation enhances the solubility of charcoal in water, which can subsequently become mobilized and integrated in

the humic fraction pool. It appears that the recalcitrance of charcoal towards oxidative decomposition is enhanced with increasing condensed aromatic structures with lower hydrogen/carbon and oxygen/carbon ratios (Preston and Schmidt, 2006).

1.2.6 Nutrient cycling

Charcoal is an important by-product of forest fires, which can alter important nutrient cycles. In boreal forest soils, researchers have shown that the application of ammonium alone had no effect on nitrification (De Luca et al., 2006). However, De Luca et al. (2006) found that forest fire charcoals resulted in an increase in gross nitrification, accompanied by a decrease in the concentration of soluble phenolic compounds. This serves as indirect evidence that charcoals stimulate nitrification through its capacity to adsorb plant phenolic metabolites (MacKenzie and De Luca, 2006; Wardle et al., 1998), and thus relieving inhibitory processes on nitrogen cycling. In agreement, Gundale and De Luca (2006) showed that charcoal produced at both 350°C and 800°C had ability to adsorb catechin (+/-), an allelochemical. In comparison between the two charcoal types, the high temperature char resulted in greater adsorption of catechin (+/-). Secondly, the total carbon content was greater in the high temperature charcoal, which suggests that nitrogen, phosphorus, sulfur, hydrogen, and oxygen were preferentially volatilized during heating at this temperature. Thirdly, the low temperature charcoal contained a significantly higher concentration of total and soluble phenols.

Research has shown that nitrogen dynamics are affected by the charcoal's degree of carbonization. Rondon et al. (2007) showed that the biomass and nitrogen uptake of non-nitrogen fixing common beans decreased due to the addition of *Eucalyptus* charcoal with a 33% VM content. Likewise, Gundale and De Luca (2007) reported that addition of 350°C char alone

caused a reduction in inorganic nitrogen, in contrast to the observed increase in nitrification with the addition of wildfire charcoal (De Luca et al., 2006). However, nitrogen mineralization and nitrification was enhanced when charcoal was added with glycine, a readily available nitrogen source. In the same study performed by Gundale and De Luca (2007), the 350°C charcoal diminished the growth of *Koelaria macrantha*, whereas as wildfire charcoal improved its growth. These scientists suggested that the forest fire charcoals may have derived under different formation conditions, particularly greater oxygen, temperature, and leaching. Though the mechanism for this discrepancy is unknown, the negative effect of low temperature chars is possibly due to nitrogen immobilization. Additionally, plant toxicity cannot be eliminated since the low temperature charcoals contained soluble phenols (Gundale and De Luca, 2006), which can potentially have a toxic effect on plants and microbes. In contrast to the low temperature char, the sorption of inhibitory phenols by the forest fire charcoal might explain its positive effect on the growth of *K. macrantha*. Further information is needed for a more complete understanding of how the production of charcoal influences its ecological significance.

1.2.7 Microbial dynamics

In addition to being an adsorbent, charcoal is also capable of supporting microbial life (Pietikainen et al., 2000). Researchers have proposed that soil microbes play a key function in the sustainability of soil fertility in Terra Preta. Kim et al. (2007) produced an oligonucleotide fingerprint grouping of 16S rRNA gene sequences, which showed that Terra Preta soils had approximately 25% greater species richness than the pristine forest soils of the Western Amazon. In agronomic applications, Rondon et al. (2007) showed that the addition of 33% VM Eucalyptus charcoal resulted in a 22% increase in nitrogen derived biological fixation for soils cropped with beans capable of nitrogen fixation. Furthermore, the colonization of roots by

mycorrhizae and biomass production were greater for nitrifying beans relative to non-nitrifying beans in soils amended with charcoal. Steiner et al. (2008) reported that increasing additions of charcoal (50, 100 and 150 g per kg soil) to a highly weathered Amazonian upland soil resulted in a significant, linear increase in basal respiration, microbial biomass, and population growth. Additions of smoke condensates caused a dramatic increase in each parameter, which suggests that the condensate is composed of easily degradable substances.

1.2.8 Agronomic value of charcoal

Previous investigations have shown both positive and negative effects of charcoal on plant growth (Table 1). A team of Australian researchers performed two greenhouse studies to compare the effect of two charcoal feedstocks on the growth of radish (Chan et al., 2007; Chan et al., 2008). In their first pot study, Chan et al. (2007) found that the addition of green waste biochar (100t/ha) did not increase the yield of radish. These researchers suggested that biochar induced nitrogen limitations, similarly to others who had observed a negative plant response to low temperature or high VM charcoals (Gundale and De Luca, 2007; Rondon et al., 2007; Deenik et al., 2010). In contrast, a second study showed a positive effect of poultry litter biochars produced at 450 and 550°C, which resulted in an increase in radish yield, which was observable at 10 t/ha. The authors attributed this to increased nutrient availability, and specifically that of nitrogen. When combined with nitrogen fertilization, the lower temperature poultry biochar resulted in significantly more radish production than the biochar produced at a higher temperature. Chan et al. (2008) concluded that the feedstock and pyrolysis temperature are important considerations for determining the value of charcoal as a soil amendment. It appears that the green waste char, inherently nutrient-poor, was less suitable for agriculture as compared to the poultry litter rich in nitrogen and phosphorus.

Several reports indicate that there is a synergistic interaction between fertilizer and charcoal (Chan et al., 2007; Chan et al., 2008; Steiner et al., 2007). Particularly, charcoal appears to increase the efficiency of nitrogen-phosphorus-potassium fertilizers, as yields of fertilized plants can be further enhanced with the additions of charcoal. A study by Steiner et al. (2007) showed that the charcoal derived from a secondary forest slowed the loss of available nutrients in comparison to soils receiving fertilizer alone. Furthermore, the fertility of charcoal-amended soils was sustained for a longer period of time, but only when additional nutrients were supplied. This makes charcoal an unlikely fertilizer source by itself, but shows that it can be instrumental in reducing nutrient losses (i.e. nitrogen, phosphorus, potassium, magnesium, and calcium) when added in concert with high-yielding, readily mineralizable fertilizers such as chicken manure (Steiner et al., 2007). Other positive effects included an increase in soil pH, reduction in exchangeable aluminum, decline in tensile strength, and improvement in field capacity (Chan et al., 2007; Chan et al., 2008). The problem with these studies is they do not characterize their charcoal and therefore mechanisms for improvement cannot be proposed.

Table 1. Differential effects of charcoal according to production temperature

Study	Charcoal feedstock	Production Temp	Crop	Rate	Positive effect on biomass	Negative effect on biomass
Lehmann et al., 2002	secondary forest	?	Cowpea	10%	almost 50% increase	
Effects of charcoal production temperature						
Chan et al., 2007	green waste	450°C	Radish	2%		50% decrease
Chan et al., 2008	Poultry	450°C	Radish	2%	30% increase	
Chan et al., 2009	Poultry	550°C	Radish	2%	almost 50% increase	
Gundale and De Luca, 2007	Wildfire	High temp	*K. Macrantha*	2%	120% increase	
Gundale and De Luca, 2007	Douglas-fir	350°C	*K. Macrantha*	2%		36% decrease
Gundale and De Luca, 2007	ponderosa pine	350°C	*K. Macrantha*	2%		25% decrease

1.2.9 Rationale for further research

Much of the literature describes the positive effects of charcoal for the enrichment of soil fertility (Chan et al., 2008, and biology; Steiner et al., 2007; Nguyen et al., 2008; Cheng et al., 2008). However, other reports show a negative effect of charcoal on plant growth, possibly due to nitrogen immobilization or plant toxicity (Gundale and De Luca, 2007; Chan et al., 2007; Rondon et al., 2007; Deenik et al., 2010). It cannot be ignored that charcoal represents a vast array of carbon materials. Its properties differ according to the feedstock, or type of organic material used for charring; environment under which the char was produced; and additions made during the charring process (Meszaros et al., 207; Antal and Gronli, 2003; Glaser et al., 2002). The literature clearly indicates that the type of feedstock and production conditions ultimately affect its suitability as a soil amendment, as well as its ecological significance. Our recent studies show that VM content in charcoal has a significant impact on plant growth (Deenik et al., 2010). We found that charcoal with a higher VM content, or less carbonized, was detrimental to plant growth. In contrast, the lower VM content, or more carbonized, charcoal enhanced plant growth when combined with nitrogen fertilization. We believe that the negative effects were caused by the stimulation of microbial activity and nitrogen immobilization due to the presence of bioavailable compounds for microbial consumption in the high VM charcoals.

Much of the interest in charcoal as a soil amendment also revolves around its positive effect on nutrient retention capacity, or cation exchange capacity, as well as carbon sequestration. Scientists propose that charcoal is effective for two reasons. First, the charcoal particle surfaces become increasingly oxidized with time. Functional groups are then available to retain nutrients, particularly due to its development of cation exchange capacity. Secondly,

this effect can be prolonged for centuries due to the stability of charcoal and its resistance to degradation. Previous research has shown that feedstock and production conditions affect the chemical properties of charcoal, such as VM content (Antal and Gronli, 2003; Meszaros et al., 2007), but few studies have examined its effects on the charge characteristics of charcoal. Based upon the findings of Cheng et al. (2006), who showed that charcoals which contained multiple functional groups and carbon forms underwent quick oxidation, we predict that high VM charcoals will develop cation exchange capacity in soils more rapidly than low VM charcoals. However, high VM content will be less stable than low VM charcoals, and also result in an initial stimulation of microbial activity.

1.2.10 Research goals and objectives

Although we have observed that VM content in charcoal affects plant growth, we do not fully understand the mechanisms that govern its behavior. The primary goal of our research is to characterize the VM content in charcoal, in order to improve our understanding of how charcoals with varying VM content affect soil processes. Ultimately, this information will improve our prediction capabilities of how plants and microbes respond to soils amended with various charcoal types.

This thesis research is presented in three chapters. In all the chapters, we study charcoals that differ in VM content and feedstock. The first chapter sets out to characterize the chemical properties of different charcoals. We describe differences in chemical structure and composition and relate these differences to the behavior of the charcoals in soils. The second paper focuses upon the effect of different charcoals on microbial and nitrogen dynamics. Results from this study aid our explanation of plant responses in our previous greenhouse

studies (Deenik et al., 2010). Finally, the third paper investigates the effect of various fresh and incubated charcoals on soil charge characteristics. Particularly, we examined the variable charge of different charcoals during an aging study, and then determined the effect of these charcoals on the soil's charge fingerprint.

2. Characterization of charcoal volatile matter content as influenced by the degree of carbonization and feedstock

2.1 Introduction

In the first decade of the 21st century, soil scientists have devoted increasing attention to the potential benefits of applying charcoal (e.g. black carbon, biochar) to soil (Laird, 2008; Lehmann, 2006; Marris, 2006). Among these benefits, the initial increases in crop yield have often been cited (Glaser et al., 2002; Chan et al., 2007). In a greenhouse study, Chan et al. (2007) determined that the combination of green waste charcoal and nitrogen fertilization dramatically increased the yield of radish. The authors attributed the improvement in yield to the increase in soil pH, exchangeable cations, and a reduction in tensile strength. However, without the addition of nitrogen, charcoal resulted in a reduction of radish yield. In a second study, Chan et al. (2008) found that poultry litter charcoal, unlike the charcoal derived from green waste, resulted in an increase in radish yield without the addition of nitrogen. This effect was more pronounced with the addition of a lower temperature poultry litter charcoal. These researchers also reported the same enhancements in soil quality observed in their previous study. While this team speculated that differential effects among charcoals were due to variations in their chemical makeup, they did not characterize the charcoal and thus could not specify the differences.

Gundale and De Luca (2007) performed one of the early investigations of the effects of charcoal production temperature on soil processes and plant growth. In this study, *Koeleria macrantha*, a perennial grass that grows well after fire disturbances, was negatively affected by ponderosa pine and Douglas-fir charcoals made at 350°C in a laboratory. However, its growth was enhanced by the addition of wildfire charcoal, presumably generated at a higher

temperature. In another study, Rondon et al. (2007) showed that a high VM (33%) charcoal resulted in a decrease in biomass and nitrogen uptake of non-nitrifying beans. Though they measured VM content, these researchers did not interpret VM content in the discussion of their results. Our previous work also shows that charcoals can have differential, short-term effect on plant growth, particularly those varying in their volatile matter (VM) content (Deenik et al., 2010). A high VM content (22.5%) macadamia nutshell charcoal had a significantly detrimental effect on lettuce and corn growth in an Ultisol and Andisol, with and without nitrogen. In contrast, a low VM content (6.3%) macadamia nutshell charcoal showed a substantial improvement in corn growth when combined with nitrogen fertilization. An analysis of the charcoals with gas chromatography-mass spectrometry (GC-MS) provided evidence that the high VM charcoal contained phenolic compounds and other products, which could potentially (1) stimulate microbial growth and the immobilization of nitrogen or (2) cause toxicity for plants and microbes.

The VM content is a charcoal property that describes its degree of carbonization. For instance, less carbonized biomass has a relatively higher VM than more carbonized materials. The VM content is measured weight loss when charcoal is heated to 950°C for 6 minutes, and generally ranges from 10 to 40% (Antal et al., 2003). At 950 °C, any remaining volatile matter of charcoal is evolved and almost pure carbon remains. Light organic compounds and gases are predominantly lost from charcoal below 600°C, whereas above 600°C the loss of VM predominately consists of water, carbon dioxide, carbon monoxide, hydrogen and methane evolution (Antal and Gronli, 2003).

Though recognized as an important property, the VM content of charcoal has not been well characterized. Antal and Gronli (2003) stated that with increasing peak temperature, the

VM content is driven off from the charcoal. Meszaros et al. (2007) reported that high VM charcoals evolved organic volatile products, including aliphatic and aromatic compounds (e.g. phenol and toluene). It is apparent that the amount of VM in charcoal is dependent upon its degree of carbonization, but may reflect the recondensation reactions of evolved tarry vapor with the charcoal during pyrolysis (Meszaros et al., 2007). The elemental changes that occur in the biomass during pyrolysis have been well documented in the literature. We know that plant residues undergo a series of transformations during the charring process as the peak charring temperature increases in a continuum. Below 220°C, the formation of water constitutes most of the weight loss from cellulose. While oligosaccharides are preserved, unsaturated single carbon-to-carbon bonds and carbonyl groups form due to the loss of water. However, up to 250 °C, carbon dioxide and carbon monoxide are also evolved. Above 250 °C, char is characterized by the appearance of phenol and furan structures. As heating temperature exceeds 290 °C, charcoal becomes dominated by alkyl furans, benzenoid aromatics, and condensed aromatics. An increase in temperature from 300 to 500 °C results in an increase in the amount of fixed-carbon as the relative oxygen content decreases. Alkyl-aromatic groups and oxygen-containing functional groups, including hydroxyl, carboxyl, carbonyl, ether, and lactone structures, can populate the surfaces of char depending upon its formation temperature. However, at 650 °C, hydroxyl, C=O, and aliphatic carbon-to-hydrogen groups are mostly absent. Most aromatic carbon-to-hydrogen groups are degraded by 750 °C, and by 950 °C, charcoal resembles graphite. Porosity, surface area, and adsorption properties also increased with increasing formation temperature (Antal and Gronli, 2003).

A number of techniques have been used to characterize the chemical structure and composition of charcoals, including fourier transform infrared spectroscopy (FTIR), ^{13}Carbon nuclear magnetic resonance (^{13}C NMR), and multiple MS analyses. FTIR is a useful method to

characterize charcoal composition (Varhegyi et al., 1998). FTIR spectra show that charcoal largely consists of aromatic carbons and oxygen-containing functional groups, such as hydroxyl, carboxyl, carbonyl, ether, and lactone structures (Antal and Gronli, 2003). FTIR has also been used to detect structural changes in the non-aromatic functional groups during the early stages of carbonization, as well as during the abiotic oxidation of the charcoal in soil (Ishimaru et al., 2007; Cheng et al., 2008). Ishimaru et al. (2007) reported that a large proportion of the oxygen-containing functional groups, as well the cross-linking of polyaromatic structures by ether and carboxylic groups, decompose and volatilize between 500 and 600°C. As a result, FTIR has shown that stronger carbonization yields less oxygen-containing functional groups and aliphatic carbons, but more aromatic carbon (Antal and Gronli, 2003; Varhegyi et al., 1998). While the charring process can potentially eliminate all functional groups depending upon the production conditions, natural oxidation of the fresh charcoal as it ages in the soil can lead to the formation of oxygen-containing functional groups (Cheng et al., 2008). Specifically, a long-term study showed that the aliphatic carbons and phenolic carbons declined to a very low proportion within ten years, while carbonyl groups increased by three-fold (Nguyen et al., 2008).

^{13}C NMR is a second method that can characterize charcoals and identify elemental and structural differences. There is also opportunity to link the degree of charcoal carbonization with the structure detected by NMR. Previous authors have used NMR to characterize charcoal, but few have comparatively studied charcoals of varying degrees of carbonization. Hamer et al. (2004) showed that their charcoal was dominated by aryl carbon but also contained alkyl carbons, and did not find major differences between maize and rye residue feedstocks. Cheng et al. (2006) too showed that charcoal examined in their study had alkyl carbons, which subsequently declined during an incubation. In contrast, Braida et al. (2009) reported that their charcoal was composed primarily of aromatic carbon with no significant aliphatic resonance. It

appears that the degree of carbonization is related to the presence or absence of alkyl carbon and oxygen substituting alkyl carbon groups, but further research is needed to validate this.

Though both FTIR and NMR techniques are capable of describing the structure and bonds in charcoal materials, neither can describe the presence of specific compounds. To address this shortcoming, we used gas chromatography-mass spectrometry (GC-MS) to identify extractable molecular groups. We hypothesize that this will provide valuable information regarding the presence or absence of bioavailable compounds, which could result in the stimulation of microbial activity and immobilization of nitrogen. In a recent study, Kaal and Rumpel (2009) used pyrolysis-GC-MS to demonstrate that partially charred materials produced phenols, n-alkanes, and n-alkenes upon pyrolysis, which generally decreased with increasing temperatures. Meszaros et al. (2007) also identified aliphatic and aromatic (e.g. benzene, phenol, toluene) decomposition products in high VM charcoals using thermogravimetry-mass spectrometry.

The first objective of the present study is to use FTIR, NMR and GC-MS to characterize charcoals with different VM contents and feedstocks. Secondly, we aim to relate differences in chemical structure and composition to previous observations of charcoal behavior in soil. Results from this study will permit a better characterization of VM content and provide insight regarding its potential short-term effects on soil. We hypothesize that VM content is quantifiable and contains phenolic compounds and hydrocarbons. These compounds are presumably either bioavailable or toxic, both of which would result in short-term reductions in plant growth in soil amended with high VM charcoals.

2.2 Materials and methods

2.2.1 Charcoal collection

We characterized charcoals from two feedstocks, including corncob husks (*Zea mays*), collected from Pioneer Seed Company on Oahu; and kiawe wood (*Prosopis pallida*), which is a leguminous tree found throughout the Hawaiian Islands. The corncob charcoals were produced using the Flash Carbonization® process developed at the Hawaii Natural Energy Institute of the University of Hawaii. This process involves the ignition and control of a flash fire at about 1 MPa within a packed bed of biomass. Heat released by the fire triggers the transformation of biomass into biocarbon with yields that can quickly reach the thermochemical equilibrium "limit" (Antal et al., 2003). In a typical run, peak temperatures after 40 minutes ranged from 300°C in bottom section of the reaction canister to just below 800°C in the upper section. We obtained three charcoal batches distinct in their thermal alteration, and specifically in their volatile matter content. The first type contained 63% volatile matter content; the second, 23%; and the third, 7%. Thus, the corncob charcoals represent a spectrum of carbonized materials, with the 63% volatile matter content being the least carbonized in contrast to the most carbonized with 7% VM. The kiawe charcoal was produced using traditional methods by a Maui-based barbeque charcoal company, and contained 23% volatile matter content. In addition to the corncob and kiawe charcoals, we also obtained activated charcoal (CAS # 7440-440) from Fisher Scientific for FTIR and GC-MS analyses only. Finally, we obtained samples of the raw feedstock materials for the corncob husks and kiawe wood prior to carbonization for GC-MS analysis only. All materials were ground and passed through a 2 mm sieve.

In order to determine whether the extraction of charcoal with acetone successfully removed all soluble compounds detectable by GC-MS, we performed a prior acetone-extraction on the 23% volatile matter corncob charcoal. We took 1.0 g of charcoal and added 10 ml of 90% acetone in 50 ml centrifuge tubes. Samples were shaken for 30 minutes, and the extract was passed through a 0.45 micron cellulose filter under vacuum. One hundred milliliters of deionized water was passed through the remaining extracted-charcoal at 10 ml increments in order to leach any remaining acetone. The extracted charcoals were then collected and placed in an oven at 105°C for 24 hours prior to analysis by GC-MS.

The23% VM corncob and kiawe charcoals and 7% VM corncob charcoals were also extracted with water, followed by the analysis of the water extracts with NMR. Water extractions were performed by adding 30 ml of deionized water to 3 g of charcoal. The samples were shaken for 30 minutes and filtered through a 0.45 µm nitrocellulose membrane. Water extracts were collected for analysis.

2.2.2 FTIR

We performed FTIR in order to examine the chemical bonds of functional groups in the charcoals. An FTIR-attenuated total reflectance (ATR) technique was employed to qualitatively analyze the functional groups in the 23% and 7% volatile matter corncob charcoals, 23% volatile matter kiawe charcoal, and the activated charcoal samples. Spectra were recorded within the 4,000 cm^{-1} to 400 cm^{-1} range with a resolution of 16 cm^{-1} by obtaining 256 scans on a Thermo Nicolet 380 spectrometer with the Smart Performer accessory. The interpretation of the FTIR spectra was intended for qualitative analysis only.

2.2.3 NMR

We used a 200 MHz Bruker DSX spectrometer (^{13}C frequency 50 MHz) equipped with a 4mm magic angle spinning (MAS) probe to obtain ^{13}C NMR spectra of the 23% and 7% volatile matter corncob and 23% volatile matter kiawe charcoals. All analyses were conducted at a rotor spinning rate of 7 kHz. We improved quantitation with a relatively high spinning rate, which moves the spinning sideband signals outside of the ^{13}C chemical shift range (i.e. 0 – 220 ppm) and prevents overlap with other signals. Cross polarization (CPMAS) were acquired by applying a 90 degree ^{1}H excitation pulse, 1ms ^{13}C contact pulse, two-pulse phase-modulated (TPPM) ^{1}H decoupling, and a 3 s recycle delay between scans.

The highly aromatic structure of charcoal makes quantitative characterization of charcoals by CPMAS NMR difficult since it inhibits efficient ^{1}H-^{13}C polarization transfer. Thus, CPMAS NMR spectra are best regarded as semi-quantitative. We overcame this limitation by using a direct polarization pulse sequence (DPMAS). We utilized a 20 degree excitation pulse, which reduced the recycle delay from 100 s to 5 s and increased the signal-to-noise per unit time by a factor of 16 relative to spectra acquired with a 90 degree excitation pulse. We acquired DPMAS spectra with ^{1}H-^{13}C dipolar-dephasing by inserting a 50 μs dephasing delay prior to the TPPM decoupling, which are devoid of signals from carbon atoms with a directly bonded hydrogen atom (C-H). Due to the behavior of aromatic model compounds, we applied a 10% intensity correction to signals in the dipolar-dephasing NMR spectra to compensate for relaxation during the 50 μs delay.

We subtracted background signals arising from carbon-containing probe and rotor components from each of the charcoal spectra so that we could quantitatively interpret the peak areas obtained by DPMAS as measurements for carbon functional groups. The quantitative reliability of all NMR experiments has been assessed by calculating the percentage of sample carbon (Equation 1) observed in the spectrum (C_{obs}), using a procedure known as spin counting (Smernik & Oades; 2000).

$$C_{obs}(\%) = 100 \times \frac{\text{signal intensity per unit carbon for sample}}{\text{signal intensity per unit carbon for glycine}} \quad (1)$$

Charcoal Structure Elucidation

We used a novel ^{13}C-^{1}H dipolar dephasing technique based upon the DPMAS sequence (described above) for a quantitative measure of the protonated versus the bridgehead aromatic carbons within the charcoal backbone structure. We estimated the average the number of aromatic carbon atoms fused together in a cluster, average number of oxygen atoms per cluster, and the average number and length of the alkyl side chains attached to each cluster of aromatic carbons, using the algorithms derived by Solum et al., (1989).

2.2.4 GC-MS

We employed GC-MS to characterize the chemical composition of the 63%, 23%, 7% VM and acetone-extracted corncob charcoal; 23% VM kiawe charcoal; activated charcoal; and raw corncob husk and kiawe wood. We extracted 1-g of each sample by sonication with acetone for 30 min. The extracts were filtered then analyzed with a Varian CP-3800 gas chromatograph

interfaced with a Varian 1200 mass spectrometer. A Factor Four VF5-MS (Varian) capillary column was used. The GC-MS ion source and transfer lines were kept at 200 and 250°C respectively, and the analysis was conducted in electron impact at 70 eV, full scan mode (50-550 u range). The NIST 2002 mass spectral library was used for compounds mass spectral identification. The maximum detectable molecular weight of a compound is 550 g mol^{-1}.

2.2.5 Prussian Blue for phenols

Prussian blue analysis to measure total phenol content followed the protocol outlined by Stern et al. (1996). Samples of 23% and 7% volatile matter corncob and 23% volatile matter kiawe charcoals were extracted with 90% acetone in a 10/1 ratio, in triplicate. One hundred microliters of the extract from each sample was transferred into 30-ml test tubes. Three ml of ferric ammonium sulfate (0.1 *M* $FeNH_4(SO_4)_2$ in 0.1 *M* HCl) was then added to successive samples at 1.0 minute intervals. Exactly 20 minutes after the ferric ammonium sulfate additions, 3.0 ml of potassium ferricyanide (0.008 *M* $K_3Fe(CN)_6$) was added to each sample, successively. Exactly 20 minutes later, the absorbance was read at 720 nm. Standards were performed with phenol.

2.2.6 Statistical analysis

Prussian blue total phenol data were log transformed and analyzed with analysis of variance (ANOVA) using SAS 9.2. Mean separation was performed by Fisher's Least Significant Difference (LSD) test.

2.3 Results

2.3.1 FTIR

We obtained FTIR spectra for the 23% and 7% volatile matter corncob charcoals, the 23% volatile matter kiawe charcoals, and the activated charcoal (Figure 1a-d). The activated charcoal spectra showed a mostly "flat" pattern (Figure 1a). However, there were slight, but observable, peaks for aromatic carbon at 1560 cm^{-1}, CH bending at 1508 cm^{-1}, C-O at 1025 cm^{-1}, and CH aromatics at 830 cm^{-1}. All the other charcoals also showed bands at these wave numbers, but with greater intensity. In comparison to the activated charcoals, the 7% volatile matter corncob showed additional bands at 2946 cm^{-1} and 2835 cm^{-1}, which were assigned to weak CH_2 and CH_3 stretching vibrations of aliphatic carbon, respectively (Figure 1b). Additionally, slight peaks were observed for C=O at 1705 cm^{-1} and C-O stretching and OH deformations of carboxyl carbon at 1250 cm^{-1}. The bands present in the 7% volatile matter corncob charcoal were also evident in the 23% volatile matter corncob and kiawe charcoals, though the bands at 1700 cm^{-1} were less pronounced (Figures 1c and d). Unlike the activated and 7% volatile matter corncob charcoals, both 23% volatile matter charcoals showed additional band intensities at 1430-1450 cm^{-1}, for CH_2 bending; at 1370-1375 cm^{-1}, for aliphatic CH_2 and CH_3 deformation; and 1110-1112 cm^{-1}, for C-O stretching or OH of carboxyl carbon. Furthermore, the bands at 1245-1250 cm^{-1} were much more pronounced than in the 7% volatile matter corncob. References for band peak assignments are provided in Table 2, by comparing absorbance peaks in our study with the available literature for FTIR peak assignments for charcoals (Lehmann et al., 2005; Varhegyi et al., 1998; Nguyen et al., 2008; Cheng et al., 2008; Ishimaru et al., 2007).

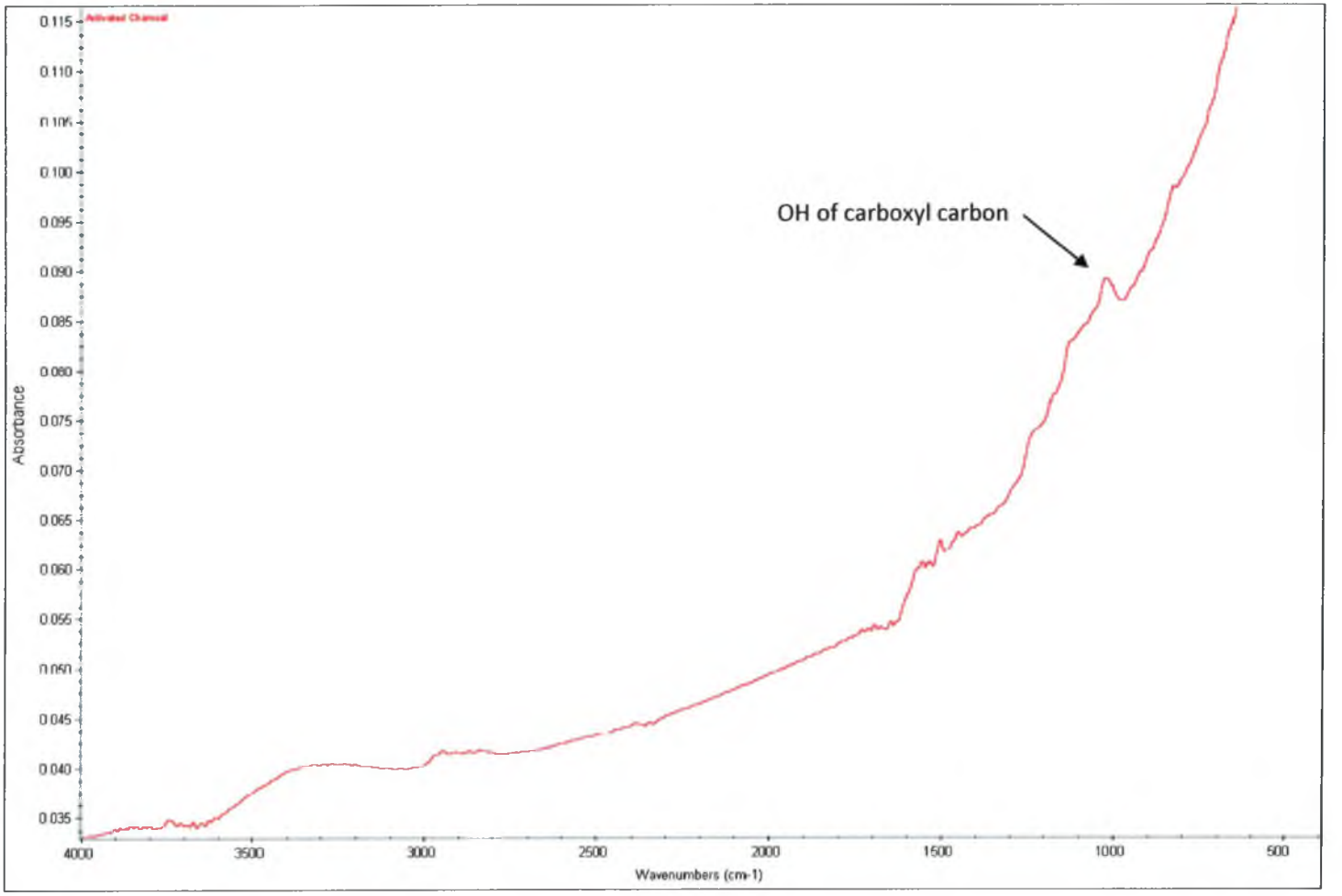


Figure 1A. FTIR-ATR spectra for the activated charcoal

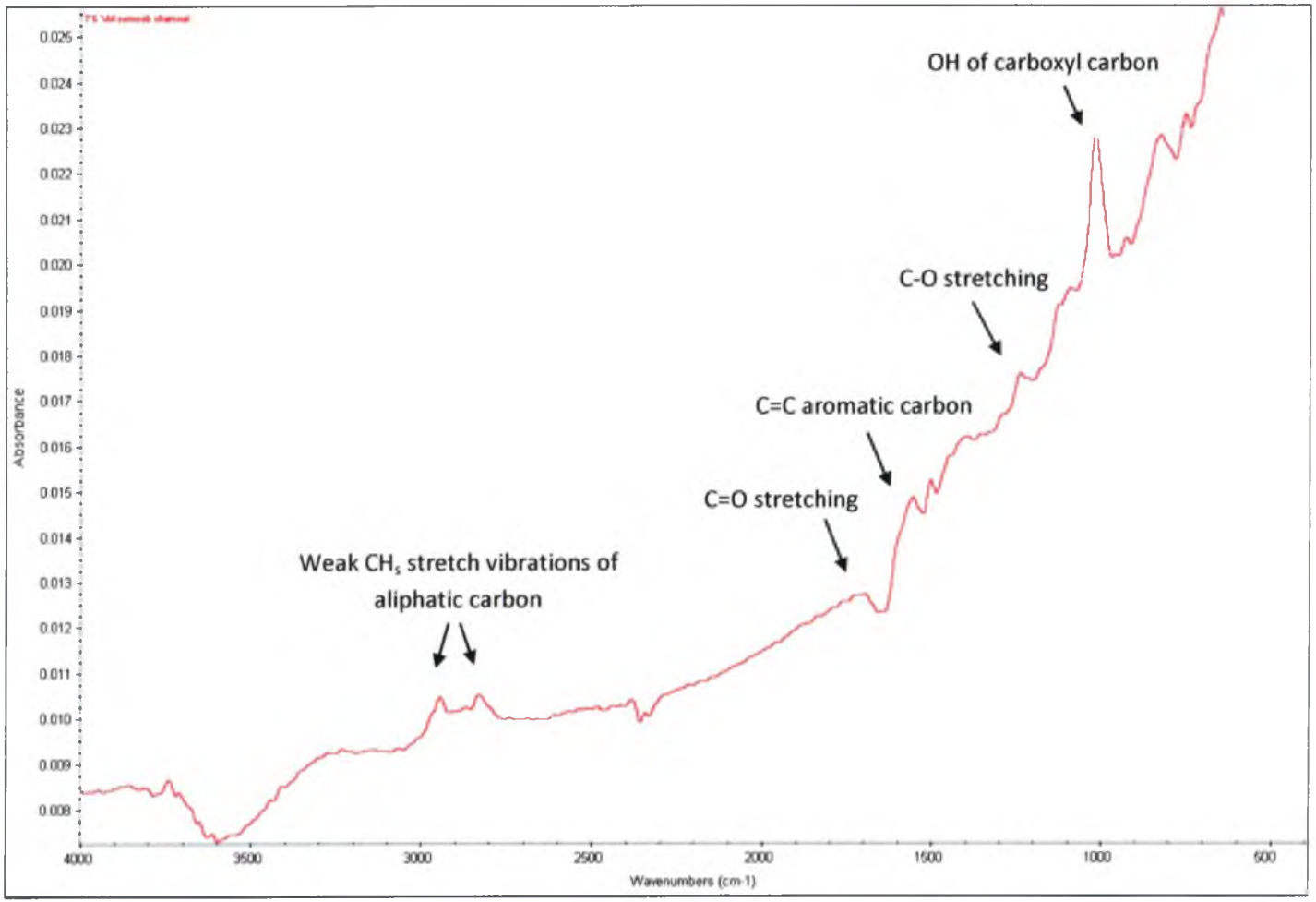


Figure 1B. FTIR-ATR spectra for 7% VM corncob charcoal

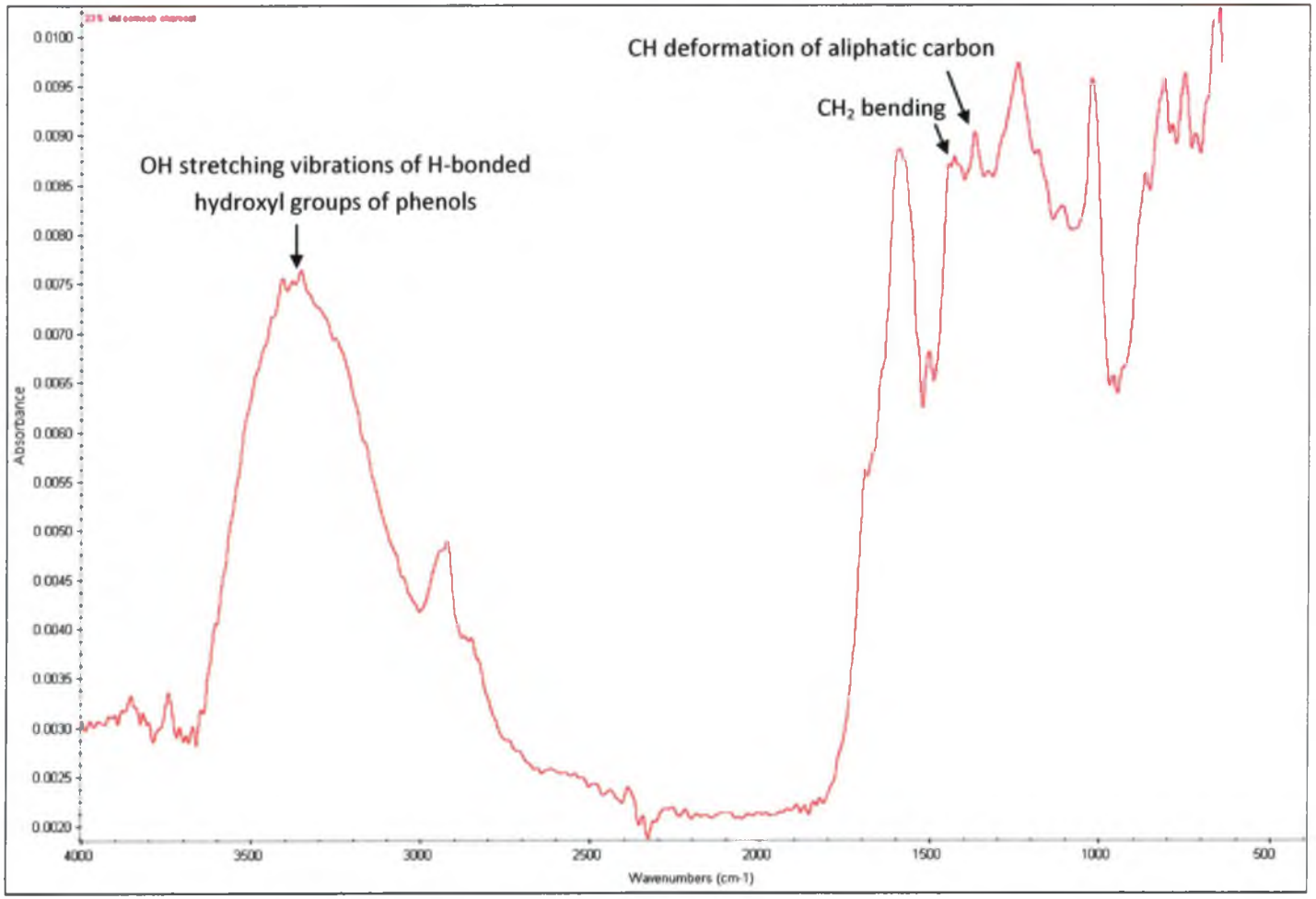


Figure 1C. FTIR spectra for the 23% VM corncob charcoal

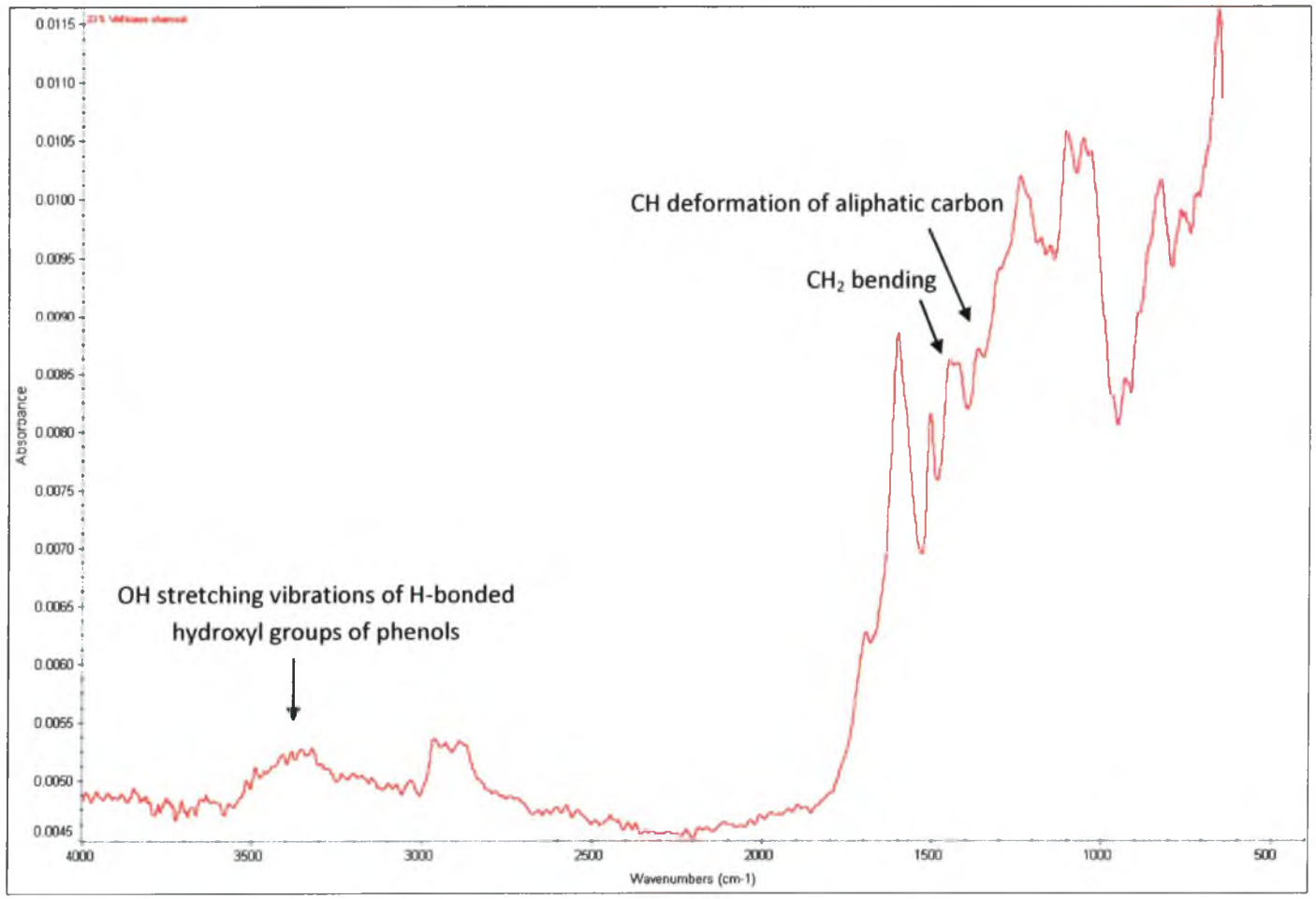


Figure 1D. FTIR spectra for the 23% VM kiawe charcoal

Table 2. Peak assignments for carbon functional groups in charcoal for FTIR

	Lehmann et al, 2005	Varhegyi et al, 1998	Nguyen et al, 2008	Ishimaru et al,2007	Cheng et al, 2008
Carbon Forms			cm^{-1}		
OH stretching vibrations of H-bonded OH groups of phenols	3411, 3339	3700-2000	3,370		3400
CH from aromatic carbon	3077	3000-2800			
weak CH_3 stretch vibrations of aliphatic carbon	2922	3000-2800	2920-2853 2920-2853		
weak CH_2 stretch vibrations of aliphatic carbon	2856	3000-2800			
COOH		shoulder at 2600			1700
C=O stretching mainly of carboxyl carbon and traces of ketones and esters	1710-1690	1700	1700	1710	
C=C of aromatic carbon	1595	~1600	1642	1590	1600
CH and NH bending	1513			1440	
CH_2 bending	1433				
CH deformation of aliphatic C	1393		1389		
C-O stretching and OH deformations of carboxyl carbon	1257	1275		1350, 1250 (ether bridges), 1150 (C-O vibrations)	1380 (carboxyl-ate), 1260
OH of carboxyl carbon	1115	~1050		1050	
C-O stretching of polysaccharides	1037		1036		
CH of aromatic carbon		900-700		900-700	

Table 2. Peak assignments for carbon functional groups, continued

	23% VM corncob	23% VM kiawe	7% VM corncob	Activated charcoal
Carbon Forms	cm-1			
OH stretching vibrations of H-bonded hydroxyl groups of phenols	3360	3400		
CH from aromatic carbon				
weak CH_3 stretch vibrations of aliphatic carbon	2927	2962, 2889	2945, 2835	
weak CH_2 stretch vibrations of aliphatic carbon				
COOH				
C=O stretching mainly of carboxyl carbon and traces of ketones and esters	1700	1700	1708	
C=C of aromatic carbon	1595	1610	1565	1559
CH and NH bending	1500	1511	1505	1508
CH_2 bending	1432	1458		1451
CH deformation of aliphatic carbon	1375	1369		
C-O stretching and OH deformations of carboxyl carbon	1245, 1112	1252, 1110	1248	
OH of carboxyl carbon	1027	1052	1026	1024
C-O stretching of polysaccharides	1027			
CH of aromatic carbon	871-754	836-767	834-751	830

2.3.2 NMR

The CPMAS NMR data showed significant structural differences between the 23% and 7% VM corncob charcoals (Figure 2). The quantitative NMR peak area and chemical assignments for the charcoals are provided by direct polarization in Table 3. We performed a mathematical subtraction of the spectra from the 23% and 7% volatile matter charcoals (inset in Figure 2), which showed that the VM content largely consists of alkyl carbon (0 – 45 ppm), oxygen-substituted alkyl carbon (45 – 95 ppm), and phenolic compounds (145 – 165 ppm). The water extractable fraction of the 23% corncob charcoal contained many different chemical groups (Table 3), particularly phenolic and alkyl carbons. In comparison to the higher VM charcoal, the low VM corncob charcoal contained more condensed aromatic carbon (120 – 135 ppm) and slightly more carboxylic groups (175 ppm). Additionally, only a small fraction of the 7% VM corncob charcoal was water soluble (1.6 - 2.8 mg C/g charcoal), and mostly comprised of light organic acids (e.g., acetic and propionic acids) (Figure 3).

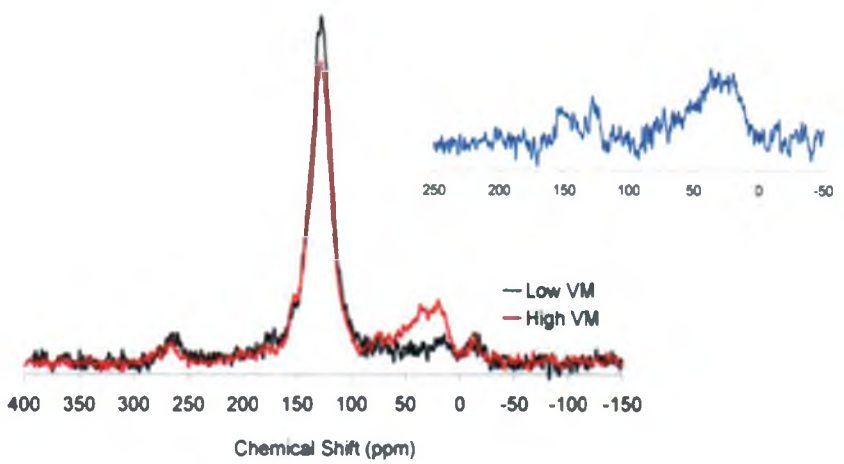


Figure 2. 13C CPMAS NMR spectra of high VM and low VM corncob charcoals. Spectra are scaled to equal quantities of carbon so that peak intensities may be compared directly. The blue spectrum (inset) represents the VM, obtained by mathematical subtraction.

Table 3. Direct Polarization ^{13}C NMR peak areas and chemical assignments for corncob and kiawe charcoals

	Alkyl	Amine / Methoxyl	O - alkyl	O_2 – alkyl / Alkene	Aromatic	Phenolic	Amide / Carboxyl	Ketone / Aldehyde
Chemical Shift region: (ppm)	0 - 45	45 - 60	60 - 95	95 - 110	110 - 145	145 – 165	165 - 190	190 – 215
	Integral areas (percentage of total spectral area)							
High VM corncob	8.8	0.9	2.2	3.2	65.9	10.6	7.1	1.3
H_2O extractable matter	9.6	1.0	2.9	2.9	63.9	15.2	2.7	1.8
Low VM corncob	5.8	1.6	3.2	3.2	72.3	9.4	3.9	0.6
H_2O extractable matter	61.4						38.6	
Kiawe charcoal	10.1	3.5	6.7	7.0	56.7	11.6	3.0	1.5
H_2O extractable matter	9.8	2.6	3.7	5.2	51.7	18.9	6.1	1.9

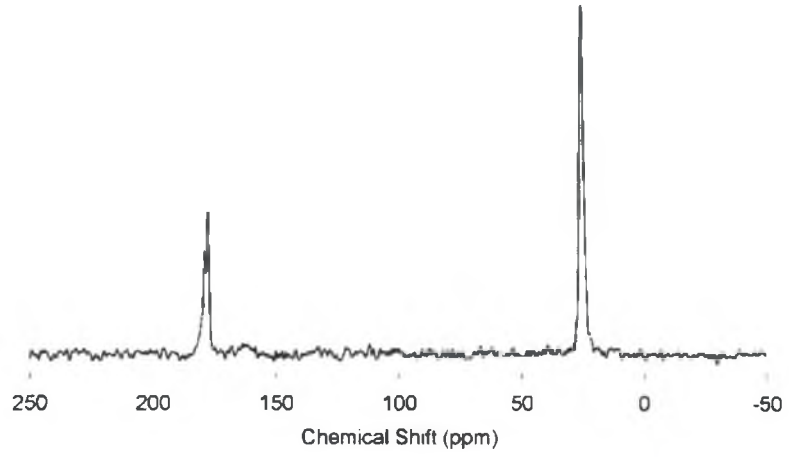


Figure 3. ^{13}C CPMAS NMR spectrum of water-extractable organic carbon from low VM corncob charcoal.

In comparison to the corncob charcoals, the kiawe charcoal was substantially less aromatic (Figure 4). Furthermore, the 23% kiawe charcoal contained more alkyl carbons (0-45 ppm), oxygen substituted alkyl carbons (45-95 ppm), and phenolics (145-165 ppm) than the other charcoals. These data suggest differences in charring severity among the charcoals. It appears that kiawe charcoal was produced at lower temperatures due to its lower aromatic, whereas the low (7%) VM corncob charcoal was produced under greater charring severity. Furthermore, we showed that a notable fraction of compounds in the high VM charcoals were extractable with water. This included alkyl, oxygen-substituted alkyl, amines, aromatic, phenolic, and ketone/aldehyde groups. Particularly, a greater percentage of the water extracts comprised of phenolic and ketone/aldehydes relative to both high VM charcoals.

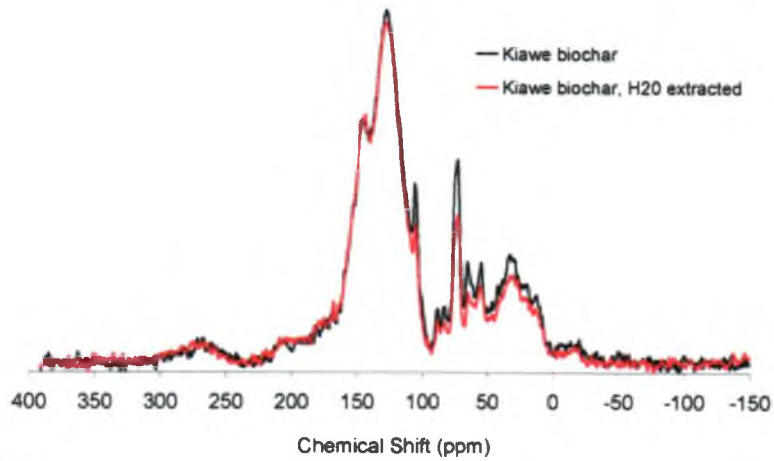


Figure 4. ^{13}C CPMAS NMR spectra of high VM kiawe charcoal before and after a water-extraction.

Structural differences can also be inferred from the DPMAS NMR measurements. We estimated that the average number of fused carbon atoms which makes up a repeating unit in the charcoal backbone was approximately 14 for the 23% VM corncob charcoal, approximately 22 for the 7% VM corncob charcoal, and approximately 8 for the kiawe charcoal. These estimates were deduced from NMR-based measurements (Figure 5) of the aromatic bridgehead carbons (signals 95 – 135 ppm in the gray NMR spectra) to total aromatic carbons (black NMR spectra). One observable difference between feedstocks is the linkages between alkyl carbons. Whereas the aromatic carbons in the corncob charcoals appear to be linked together by one to two alkyl carbons, the neighboring aromatic clusters (R) in the kiawe are linked by an alkyl with an average of four carbons in length. Using this information, we were able to configure hypothetical chemical structures for each of the charcoals (Figure 5), which illustrate the number of aromatic carbon atoms fused together in a cluster. The DPMAS and CPMAS NMR results suggest that 7% VM corncob charcoals were formed under conditions of greater pyrolysis severity, than the higher VM charcoals. This resulted in the devolatilization or carbonization of alkyl structures that were observable in the high VM charcoals.

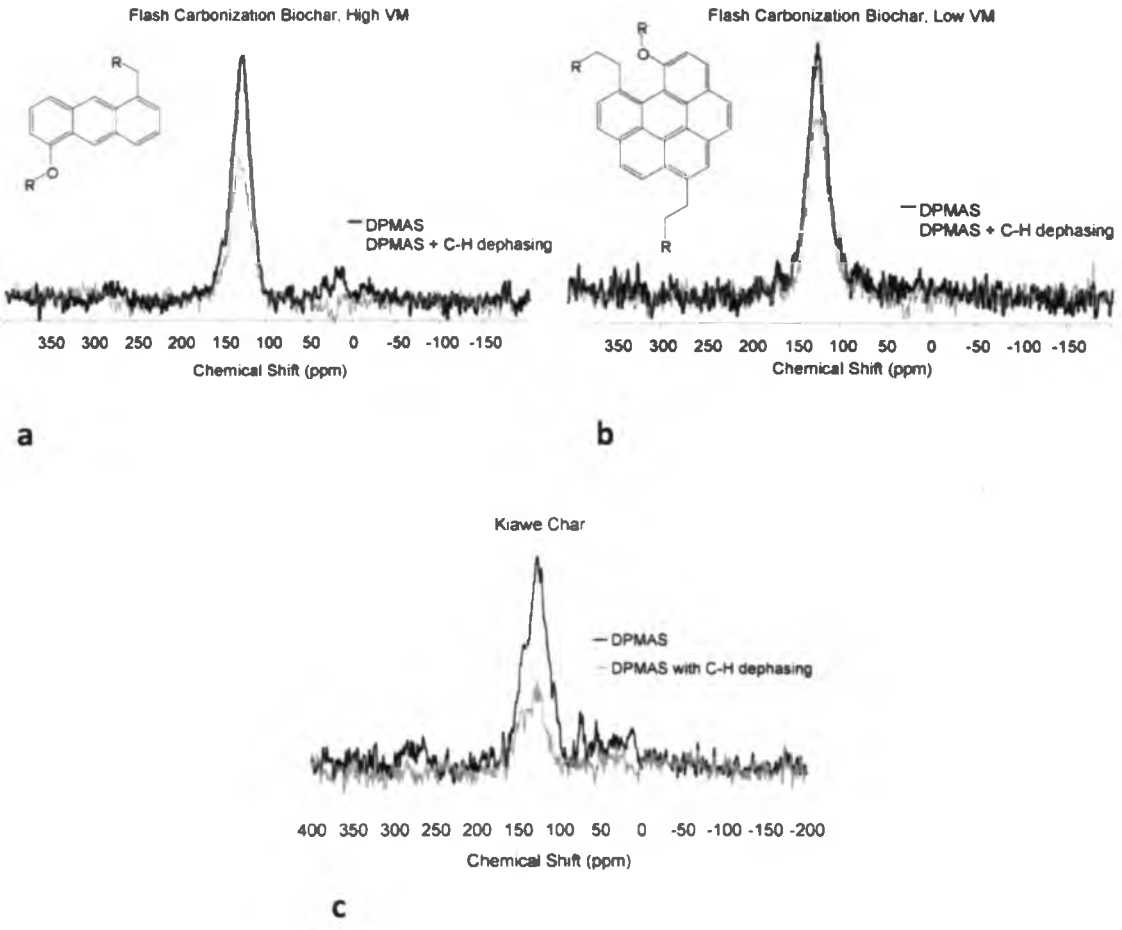


Figure 5. Quantitative 13C NMR spectra. Proposed structure of average molecular repeating units for the high VM (a) and low VM (B) corncob charcoals and high VM kiawe charcoal (c).

2.3.3 GC-MS

The GC-MS chromatograms were developed for the raw corncob husk and kiawe wood feedstocks, the 63%, 23%, and 7% volatile matter corncob charcoals; 23% volatile matter kiawe charcoals; activated charcoal; and 23% volatile matter corncob charcoal after a previous acetone extraction (Figure 6a-h). Results indicate the compounds which were extracted by acetone prior to detection by GC-MS analysis. The raw corncob contained an oxygen-substituted aliphatic (dodecylacrylate) and fatty acids (palmitic and oleic acids). In comparison, the raw kiawe contained relatively more compounds, including a phenol (dimethylethyl phenol), an oxygen-substituted aliphatic (dodecylacrylate), fatty acids (palmitic, oleic, and stearic acids), a long-chain amide (erucamide), long-chain hydrocarbons (octacosane, nonacosane, and lycopersene), vitamin E, and plant cholesterols (campesterol, stigmasterol, and sitosterol). The GC-MS chromatograms showed a range of compounds in the 63% and 23% volatile matter corncob charcoal. The 63% volatile matter corncob charcoal contained substituted phenols (ethylphenol, ethyl guaiacol, vinyl guaiacol, syringol, vanillin, and methoxyphenol), an alcohol (tridecanol), a fatty acid (palmitic acid), an oxygen-substituted aliphatic (dodecylacrylate), and polycyclic compounds (benzopyranone, fluoranthene, and pyrene). In comparison, the 23% volatile matter corncob contained relatively less compounds, which included a phenol (dimethylethylphenol), an amide of oleic acid (oleamide), a carboxylic acid (propanoic acid),an oxygen-substituted aliphatic (dodecylacrylate), and a fatty acid derivative (myristic aldehyde). In comparison, we did not detect any compounds in the 23% volatile matter kiawe charcoal within the detection range of the instrument. Likewise, the 7% volatile matter corncob, the acetone-extracted 23% volatile matter corncob, and activated charcoal showed no peaks in their chromatograms.

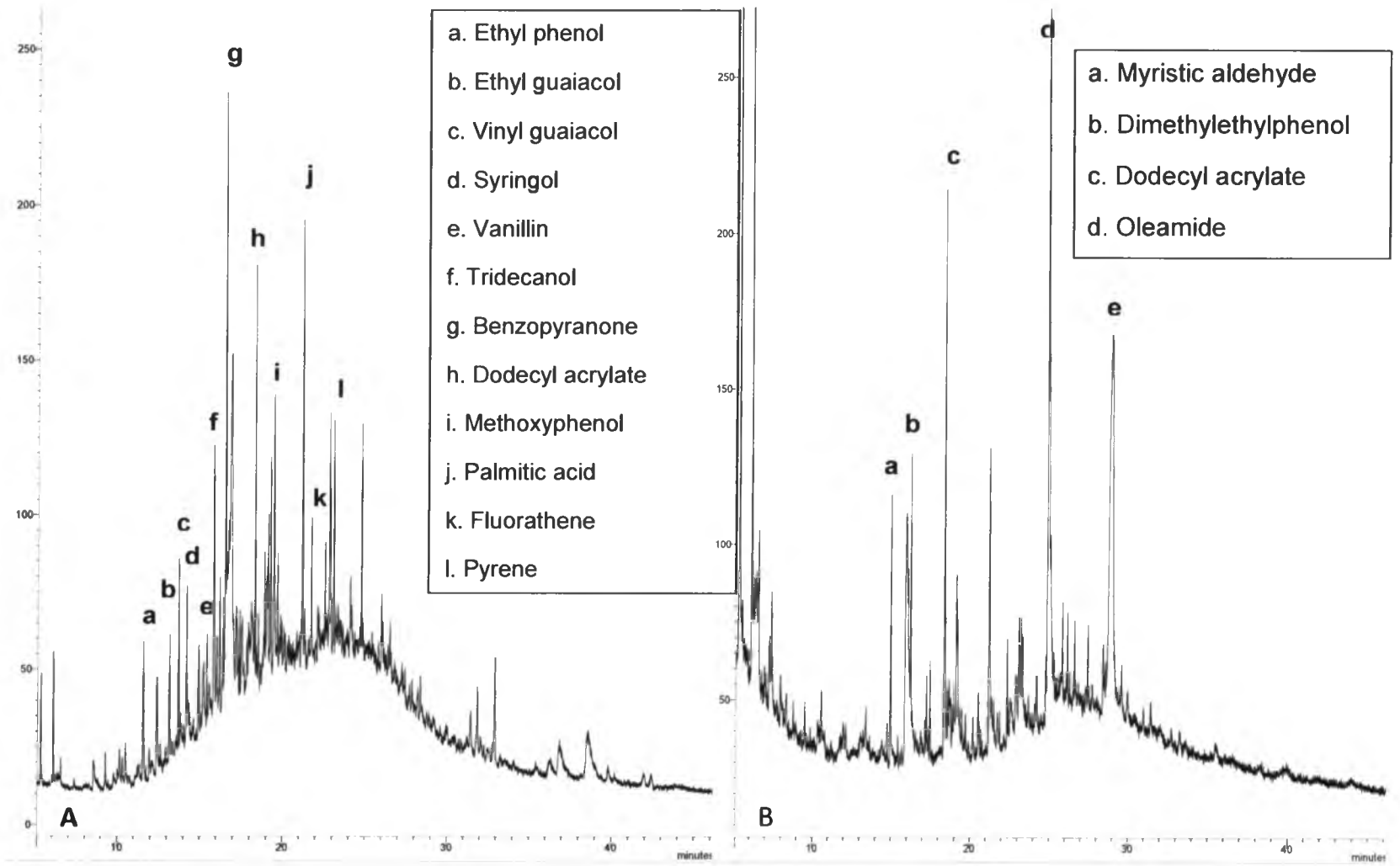


Figure 6A&B. GC-MS chromatograms. Chemical composition of the acetone extracts for (A) 63% VM content corncob charcoal and (B) 23% VM content corncob charcoals.

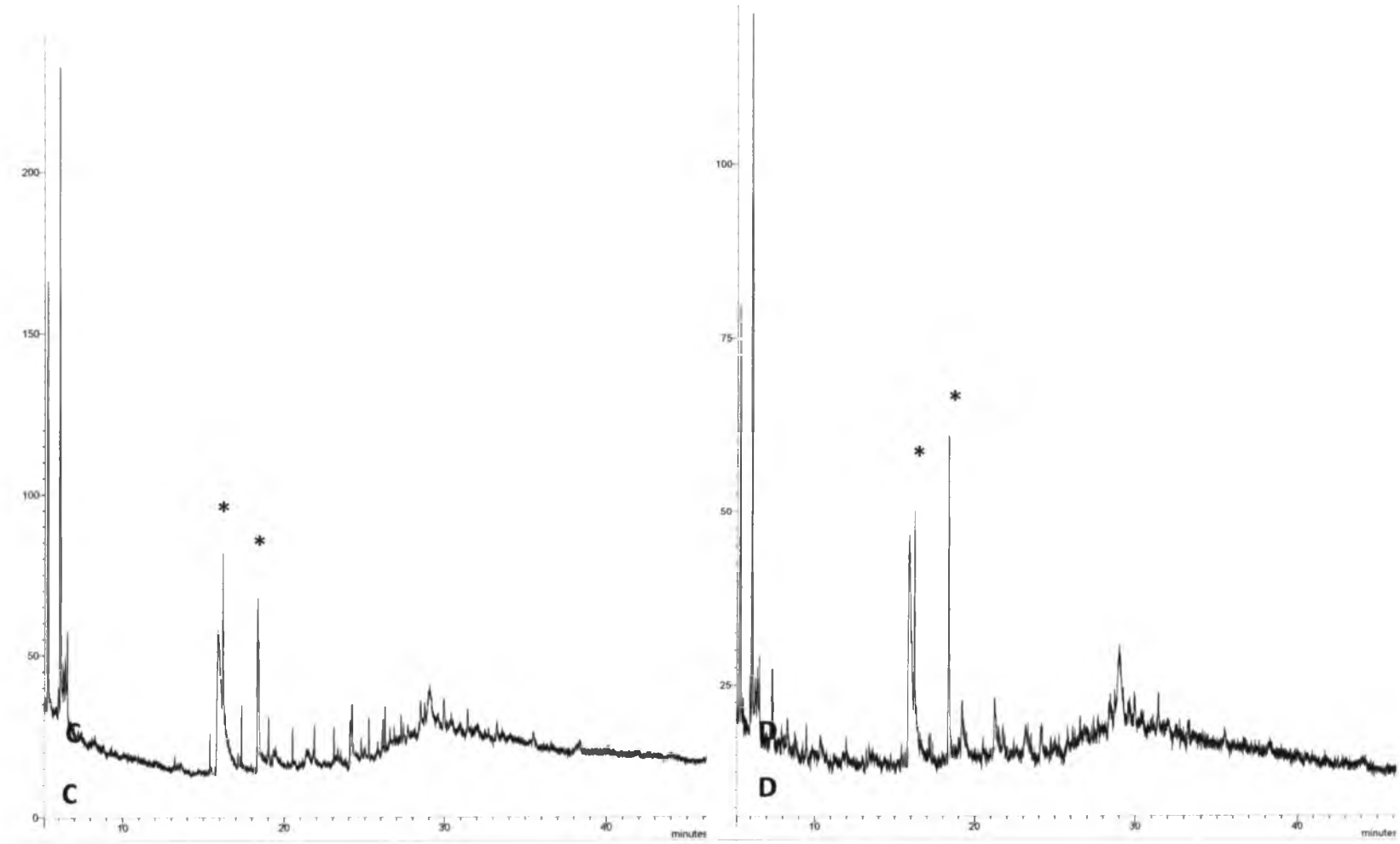


Figure 6C&D. GC-MS chromatograms. Chemical composition of the acetone extracts for (C) 7% VM corncob charcoal and (D) acetone-extracted 23% VM charcoal. Asterisks indicate background peaks.

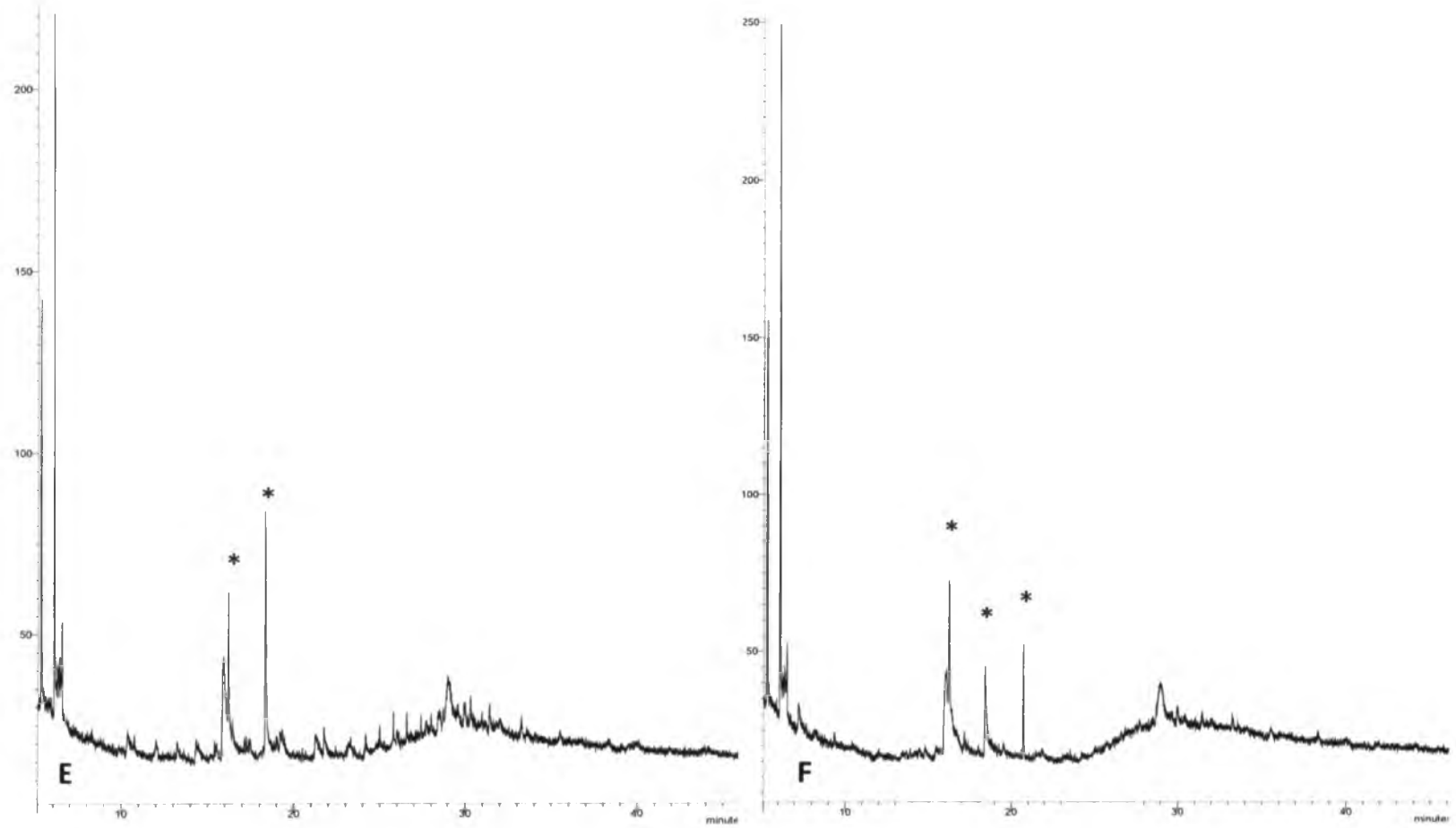


Figure 6E&F. GC-MS chromatograms. Chemical composition of acetone extracts for (E) 23% VM content kiawe charcoal and (F) activated charcoal. Asterisks indicate background peaks.

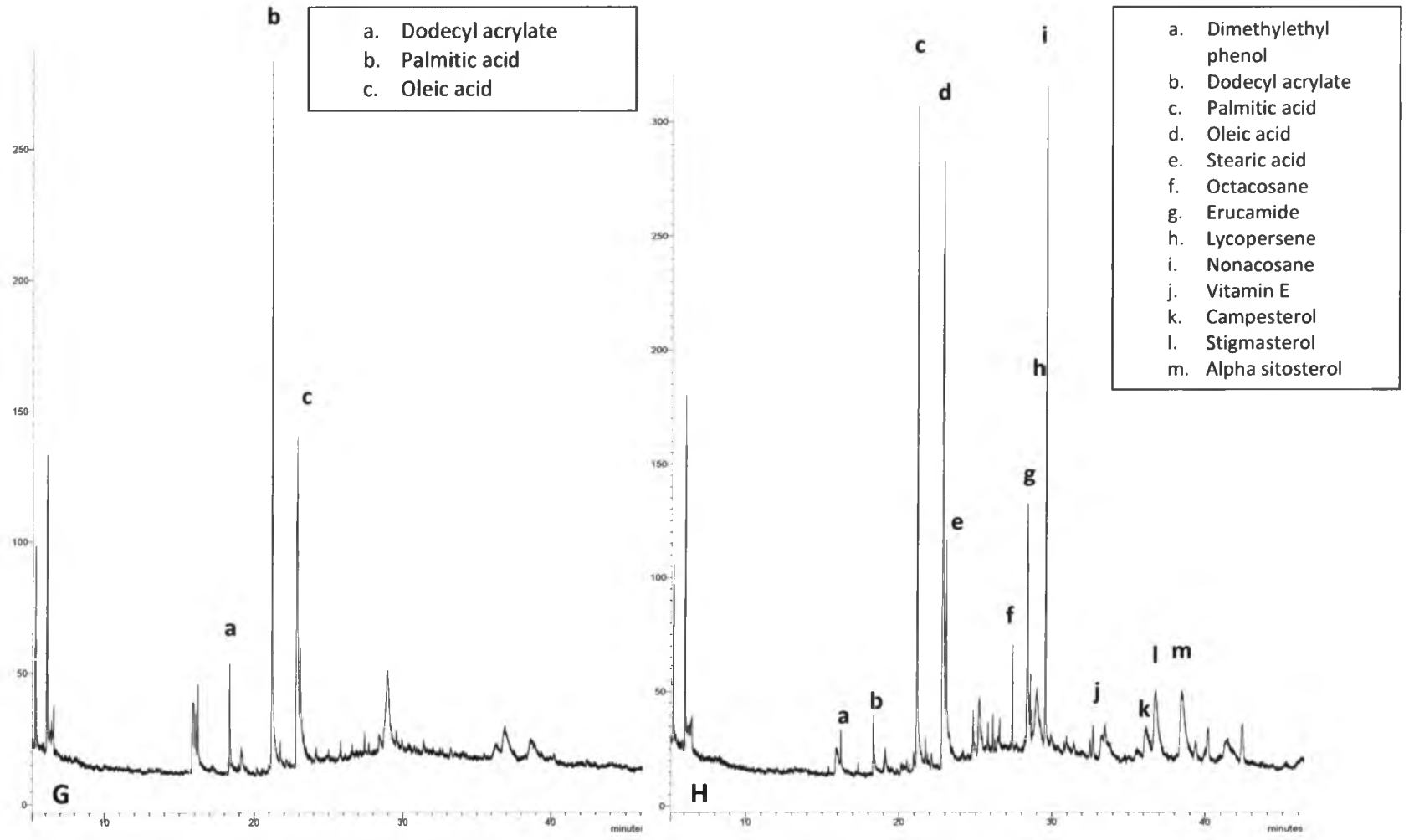


Figure 6G&H. GC-MS chromatogram. Chemical composition of acetone extracts for (G) raw corncob husks and (H) raw kiawe wood.

2.3.4 Prussian blue

Prussian blue colorimetric assay showed that the 23% and 7% volatile matter corncob and 23% volatile matter kiawe charcoals, as well as the previously acetone-extracted 23% volatile matter corncob, varied significantly in their total phenol content (Figure 7). Interesting, the 23% volatile matter kiawe charcoal contained the most extractable total phenols at 842.7 ± 15.8 μg g^{-1}, despite the GC-MS detecting no phenolic compounds. This finding suggests that the kiawe charcoal contains high molecular weight polyphenolic compounds. The 23% volatile matter corncob charcoal contained 633.3 ± 73.7 μg g^{-1}, followed by the acetone-extracted 23% volatile matter corncob with 224.8 ± 8.8 μg g^{-1} and 7% volatile matter corncob charcoal contained the lowest with 14.4 ± 3.3 μg g^{-1}.

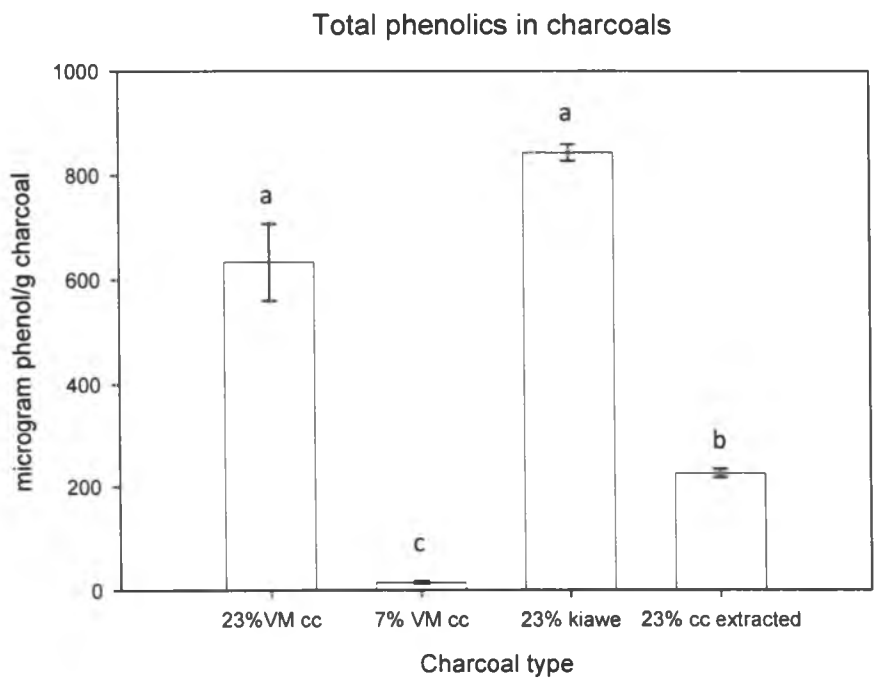


Figure 7. Total phenols for different charcoals measured with Prussian blue assay. (cc= corncob charcoal and extracted= acetone-extracted 23% volatile matter corncob charcoal). Treatments with the same letter are not statistically differ at $p<0.05$. Grouping was performed on log transformed data.

2.4 Discussion

Our results indicate that charcoals consist of a continuum of compounds depending upon VM content and feedstock. In the present study, we investigated two charcoal fractions: (1) the solid charcoal fraction and (2) the acetone-extractable fraction. For the first fraction, we used FTIR and NMR to examine differences in the chemical structure of solid charcoal. In regard to the second fraction, we extracted the charcoals with acetone and identified soluble chemical compounds with GC-MS and total phenols with Prussian Blue. We found differences in chemical structure due to VM content, while the detection of soluble compounds varied according to feedstock and VM content. Taken together, the characterization of the two fractions can provide important insight for the behavior of charcoal in soil.

2.4.1 Characterization of chemical structure of VM content in charcoal

Few prior studies have directly related VM content of charcoal to differences in chemical structure. Volatile matter content is an easily measurable property, which is dependent upon peak temperature. Since it is a property of the charcoal itself, it a reliable indicator of its degree of carbonization. Our findings showed notable differences between the chemical structures of high and low VM charcoals. Particularly, we showed that high VM charcoals contained greater amounts of alkyl, oxygen-containing compounds, and phenolic carbons. In agreement with our observations, Meszaros et al. (2007) also showed that high VM charcoals contained aliphatic, aldehydes, oxygen-containing compounds, aromatic (e.g. toluene and phenol) and carbohydrate compounds, which were evolved with increasing temperatures during their thermogravimetric analysis.

The VM content of charcoal is an especially useful property because of its relationship to carbonization temperature. By referencing the regression analysis between carbon content and production temperature provided in Figure 3b of Antal and Gronli (2003), we estimate that the 7% VM corncob charcoal was produced around 550°C; 23% VM corncob, at 375°C; and 23% VM kiawe, 325°C. Many researchers describe charcoal in terms of production temperature (Gundale and De Luca, 2007; Ishimaru et al., 2007; Cheng et al., 2006; Hamer et al., 2004). Similar to our study, other scientists have observed alkyls and oxygen-substituted alkyls in charcoals produced at 250°C (Hamer et al., 2004) and 350°C (Cheng et al., 2006). Antal et al. (1998) reported that stronger carbonization results in a decline in oxygen-containing functional groups and aliphatic CH, while Ishimaru et al. (2007) showed that the oxygen-containing functional groups and cross-linking of polyaromatic stacks volatilize with increasing production temperatures up to 600°C. Above this temperature, the predominate evolving species are H_2O, CO_2, CO, CH_4, and H_2 (Meszaros et al., 2007). Unfortunately, production temperature is not a measurable property of the charcoal. Volatile matter content, on the other hand, is an inherent property of the charcoal, which tells us about degree of carbonization and chemical structure.

2.4.2 Implications of structural differences in VM content

The VM content of charcoal is a measurable property, which our results show is related to changes in chemical structure due the charring process. The chemical structure of charcoal has direct implications for its behavior in soils. Particularly, the degree of carbonization can have a pronounced effect on the charcoal's degradability. Charcoals with a high VM content contain relatively higher alkyl carbon/aryl carbon ratio than low VM charcoals. Hamer et al. (2004) showed that maize and rye charcoals, with a 13% and 14% alkyl carbon contents and 73% and 71% aromatic carbon (respectively), were susceptible to the mineralization of carbon dioxide. These charcoals were less resistant to degradation than a charcoal derived from wood,

which had greater aromaticity (77%) and lesser alkyl carbon content (9%). Similarly, Baldock and Smernik (2002) found that the increasing conversion of alkyl carbon to aryl carbon during pyrolysis corresponded with a reduction in carbon mineralization. In our study, the direct polarization NMR data indicated that the 23% volatile matter corncob contained 66% aromatic carbon and 9% alkyl carbon, whereas the kiawe charcoal was estimated to have 57% aromatic carbon and 10% alkyl carbon. In contrast, the 7% volatile matter corncob comprised of approximately 6% alkyl carbon and 72% aromatic carbon. Based on these observations, we would predict that high VM charcoals are relatively more bioavailable than low VM charcoals. The enhanced bioavailability of high VM charcoals, due to their lesser aromaticity and greater alkyl carbon content, provides evidence in support of speculations that charcoals may be involved in nitrogen immobilization (Deenik et al., 2010; Gundale and De Luca, 2007, Rondon et al., 2007)).

2.4.3 Differences in chemical composition of extractable compounds

High VM charcoals contain partially carbonized compounds (Varhegyi et al., 1998), as well as volatiles that have recondensed upon the solid surface of charcoal (Antal and Gronli, 2003; Meszaros et al., 2007). As a polar aprotic solvent, acetone may be capable of desorbing chemicals from the charcoal surface and dissolving certain compounds. The characterization of this fraction provides important information regarding the presence of compounds that can be readily removed or dissolved from the charcoal. In our analysis of the acetone-extractable fraction of corncob charcoal, we showed that VM content influenced its composition. For instance, the 63% VM corncob charcoal contained numerous phenolic and hydrocarbon compounds. In comparison, the 23% VM corncob contained only one phenolic compound and three possible fatty acid derivatives, whereas we detected none in the 7% VM corncob charcoal.

These results confirmed our expectations that alkyl and oxygen-containing carbon compounds disappear with increasing thermal alteration.

Interestingly, our fractionation of charcoal with acetone showed differences between the high VM charcoals. Unlike the corncob charcoal of an equivalent VM content, we were unable to detect any molecular compounds by GC-MS in the 23% volatile matter kiawe charcoal. These results were in spite of its relatively high VM content and functionality (as observed by NMR and FTIR) and higher abundance of total phenols according to Prussian Blue analysis. Since the quantification of total phenols includes polyphenolic compounds, it is possible that the high VM kiawe charcoal contained compounds that (1) were not extractable by acetone or (2) were not within the detection range of the GC-MS due to their higher molecular weight or complexity. Charring lignin, a major component of wood, generates mostly condensed carbons and methoxyphenol volatile compounds with greatest yields obtained between 500 and 600°C (Knicker, 2007). In their TG-MS analysis, Meszaros et al. (2007) showed that a high VM oak wood charcoal evolved similar aliphatic products but lesser phenol, benzene, and toluene relative to a high VM corncob charcoal. In our study, we also showed differences between the high VM kiawe and corncob charcoals. Though our NMR data indicated that the high VM kiawe contained relatively more alkyl, oxygen-substituted alkyl, and phenolic carbons; no molecular compounds were solubilized and detected by GC-MS. An investigation of differences in the feedstock materials and its relationship to the chemical character of charcoal demands further investigation.

Our results highlight the need for a more complete characterization of the molecular composition of high VM charcoals. Here, we provide a simple survey of the molecular compounds which makeup a readily extractable fraction of charcoal. We were able to show

differences in the extractable compounds due to VM content and feedstock. However, follow-up studies should be conducted to further fractionate the extractable portion charcoal with stronger solvents, such as ethanol and hexane. Additionally, other techniques should be used to obtain a complete molecular characterization of the solid charcoal. In particular, thermogravimetry-mass spectrometry (TG-MS) and pyrolysis-GC-MS (Py-GC-MS) have proven to be useful in comparing the decomposition products of different charcoal types (Kaal and Rumpel, 2009; Meszaros et al., 2007).

2.4.4 Implications of the presence of extractable compounds on soil processes

Previous greenhouse studies have shown that charcoal VM content (Rondon et al., 2007; Deenik et al., 2010) and charcoal production temperature (Gundale and De Luca, 2007) affect plant growth. All studies showed that less carbonized charcoals had a negative impact on biomass production. Though Rondon et al. (2007) and Gundale and De Luca (2007) did not characterize the charcoals, Deenik et al. (2010) showed that the charcoals that diminished plant growth contained an array of easily extractable compounds which were identified with GC-MS. Since the charcoals contained hydrocarbons and numerous phenols, the detrimental effect on plants may have been due to the immobilization of nitrogen by microbes or phytotoxicity. Phenol is a known to be both a phytotoxic chemical (Wang et al., 2002) and a metabolic substrate for soil microorganisms (Saravanan et al., 2008). Prior to this study, researchers speculated that charcoals contained labile compounds. We provide evidence that charcoals indeed contain extractable compounds, which are both potentially bioavailable and toxic. Effects are likely to be temporary due to the potential solubility, and thus removal, of compounds when entering the soil environment.

2.5 Conclusion

In conclusion, we have shown that there are measurable differences in the chemical structure and composition of charcoals with varying VM contents. Structurally, the "VM content" of charcoals appears to be comprised of alkyl carbons, oxygen-substituted alkyl carbons, and phenolics. We observed molecular differences between the acetone-extractable fractions of two high VM charcoals. Thus, despite VM content being an easily measurable property that denotes major structural and molecular differences within one feedstock, its interpretation is limited when making comparisons among different feedstocks. Two charcoals with the same VM content but different feedstocks can have major differences, particularly in the molecular composition of its extractable fraction, as demonstrated here.

The combination of several techniques and a simple fractionation of charcoal with acetone have improved our understanding of the effect of production temperature on the chemical makeup of charcoal. Ultimately, we can relate the chemical structures and the composition of extractable compounds in various charcoals to the differential impacts on plant growth and soil processes observed by Deenik et al. (2010) and Gundale and De Luca (2007). High VM charcoals can contain partially carbonized carbons and extractable alcohols, fatty acids, phenols, and PAHs. Thus charcoals can contain compounds that promote microbial growth and nitrogen immobilization, as well as allelopathic compounds that hinder plant growth. In the future, we need to perform more extensive characterization of molecular composition of charcoal and to link chemical changes during increasing carbonization with inherent differences in the feedstock material.

3. The effect of charcoal volatile matter content and feedstock on microbial and nitrogen dynamics

3.1 Introduction

The sequestration of carbon and the enhancement of soil quality are among the most imminent challenges imposed upon the world's arable lands. While the former concern aims to combat global warming by offsetting anthropogenic fossil fuel emissions through the capture and storage of carbon in soils (Marris, 2006), the latter involves the implementation of practices that provide adequate food, fiber, and fuel without hindering soil health and function (Karlen, 1997). Lehmann et al. (2006) proposed that the best approach to terrestrial carbon sequestration is by the additions of biomass-derived charcoal (also biochar or black carbon), which provides the opportunity for long term carbon storage and, acting as a soil conditioner, for improved soil fertility and increased crop production. However, the stability of charcoal ultimately determines its value as a carbon sequestering material. Though charcoal is largely considered to be biochemically inert, eventual decomposition is inevitable (Schmidt and Noack; 2000).

The turnover of charcoal remains uncertain, with reports indicating both rapid (Bird et al., 1999) and slow (Shindo, 1991) decomposition. Bird et al. (1999) reported that charcoal can undergo natural degradation in well-aerated environments within 100 years, during which larger charcoal pieces are decomposed into finer particles. This is accompanied by an increase in the resistance to further oxidative degradation with time. However, these authors could not conclude whether the degradation was due to the complete mineralization of carbon by microbes or the result of solubilization into the dissolved organic carbon pool. Contrary to these

findings, Shindo (1991) determined that no significant decomposition occurred during a 40 week incubation of charred residue collected from a grassland fire in a volcanic soil.

The utilization of charcoal by microbes is inconclusive. Bruun et al. (2008) attempted to show microbial assimilation by adding charcoal labeled with ^{14}Carbon. Though the presence of char enhanced the evolution of carbon dioxide, these authors could not provide direct evidence of assimilation, perhaps due either to insensitivity of the measurement or lack of its occurrence. Another study, performed by Hamer et al. (2004), successfully demonstrated that charcoal can promote microbial growth and the mineralization of readily available compounds. Not only did this team of researchers provide evidence for the mineralization of charcoal, they also showed that co-metabolism is important for the degradation of charred materials. Specifically, the mineralization of charcoal, as measured by carbon dioxide evolution with time, was stimulated by approximately 2-fold upon the addition of glucose. Furthermore, glucose mineralization was also enhanced by the presence of charred residue, suggesting an interactive priming of charcoal. In contrast, other studies suggest that aggregation and physical protection decreases mineralization of charcoal (Lehmann et al., 2006).

In addition to mineralization, a second fate of charcoal appears to be its dissolution and subsequent transport through the soil profile. Hockaday et al. (2006) identified the presence of condensed aromatic ring structures with extensive substitution by oxygen-containing functional groups in the dissolved organic matter of soil water. The authors determined that the molecules had been transported from fire-derived charcoal in surface soils and were similar in structure to the condensed aromatic ring structures isolated in the charcoal leachates. Additionally, filamentous saprolytic fungi were also found to inhabit the 100-year char, which may play an important function in the degradation of macroparticles into water soluble molecules through

their enzymatic activity. The authors suggested that the oxidation of char, followed by the formation of water-soluble molecules, was facilitated by fungal co-metabolism. In a follow-up study, Hockaday et al. (2007) proposed that the pathway of char degradation by fungi is similar to that of polycyclic aromatic hydrocarbons. However, a greater abundance of fungal hyphae could also lead to a decline in decomposition due to physical protection by the formation of aggregates (Brodowski, 2004; Lehmann et al., 2006).

The stability and recalcitrance of charcoal against biotic and abiotic oxidation is ultimately dependent upon the properties of the charcoal, which is contingent upon production conditions and feedstock (Lehmann et al., 2006). Bruun et al. (2008) confirmed that carbon dioxide evolution was dependent upon the degree of thermal alteration. In general, the charcoal mineralization decreased with increasing production temperature. Hamer et al. (2004) and Baldock and Smernik (2002) determined that the aryl-carbon content of the charcoal influenced the susceptibility of char towards degradation. Baldock and Smernik (2002) reported that *Pinus resinosa* sapwood charred at temperature below 200°C had a higher degradability and contained a lesser aryl-carbon content as determined by NMR. The mineralization of carbon for wood heated at 150°C was 13%, in comparison to 2% for wood charred between 200 and 350°C. Similarly, Hamer et al. (2004) reported that oak wood charred at 800°C was less susceptible to decomposition than 350°C maize and rye char, corresponding to a higher aryl-carbon content and carbon/nitrogen ratio in the wood charcoal.

Gundale and de Luca (2007) showed that a relatively low temperate charcoal (produced at 350°C) reduced the mineralization of soil nitrogen and nitrification. The authors speculated that the low temperature charcoal contained a source of bioavailable carbon which stimulated nitrogen immobilization. When added with labile nitrogen source glycine, the nitrogen

limitations were relieved and mineralization and nitrification were enhanced. A later study showed that volatile matter (VM) content is an important charcoal property, which largely influences the labile carbon pool and charcoal behavior in soil (Deenik et al., 2010). Charcoals that contain high amounts of VM content are less carbonized and were shown to negatively affect plant growth. Enhanced microbial activity and nitrogen immobilization appear to explain this negative effect. Charcoal with low VM content have been shown to not adversely affect plant growth. It is speculated that low VM charcoal do not contain a readily bioavailable carbon source, thus not stimulating microbial activity.

It is evident that charcoal is susceptible to microbial degradation to various extents. It is also apparent that the charcoal properties, specifically feedstock and degree of thermal alteration, influence its stability and degradability. However, previous research has only determined the mineralization of charcoal with methods involving the measurement of mass loss and/or carbon dioxide evolution. Further information is needed to elucidate what types of carbon compounds are available for microbial degradation, whether these compounds are water soluble and how different feedstocks and VM content influence the presence and degradation of these bioavailable carbons. The objective of the present study is to determine how the chemical composition of VM affects microbial and nitrogen dynamics. We hypothesize that a higher VM content will enhance microbial growth and activity resulting in N immobilization due to the presence of a labile carbon pool.

3.2Materials and methods

3.2.1 Soil collection

The soil used in this experiment was an uncultivated, highly weathered Ultisol (Leilehua series, very fine, ferruginous, isothermic, ustic kanhaplohumult) collected from the 30-80 cm depth at the Wahiawa Correctional Facility, Mililani, Oahu Island (N21° 26'53".W157°57'52"). This soil was used in previous greenhouse studies to determine the effect of VM content on plant growth (Deenik et al., 2010). The soil was sieved through a 6 mm aperture sieve in the field, placed In sealed 18.9 liter buckets to maintain field moisture, and transported to the greenhouse facilities of the University of Hawaii at Manoa. A subsample from the soil was sent to the Agricultural Diagnostic Service Center for analysis (Table 4). In the laboratory, soils were sieved once more to pass through a 2 mm sieve and stored in closed containers at 24°C in preparation for the laboratory experiments.

3.2.2 Charcoal collection

The charcoal feedstocks used in our laboratory studies included corncob husks, collected from Pioneer Seed Company on Oahu, and kiawe wood. The corncob charcoals were produced using the Flash Carbonization process developed at the Hawaii Natural Energy Institute of the University of Hawaii. This process involves the ignition and control of a flash fire at about 1 MPa within a packed bed of biomass. Heat released by the fire triggers the transformation of biomass into biocarbon with yields that can quickly reach the thermochemical equilibrium "limit" (Antal et al., 2003). In a typical run, peak temperatures after 40 minutes ranged from 300°C in bottom section of the reaction canister to just below 800°C in the upper section. We obtained three charcoal batches distinct in their thermal alteration, and

specifically in their volatile matter content. The first type contained 34% volatile matter content; the second, 23%; and the third, 7%. Thus, the corncob charcoals represent a spectrum of carbonized materials, with the 34% volatile matter content being more partially carbonized in contrast to the more carbonized 7%. The kiawe charcoal was produced using traditional methods by a Maui-based charcoal company, and contained 23% volatile matter content. Finally, activated charcoal (CAS #7440-440) was obtained from Fisher Scientific.

Selected chemical properties of the charcoals are presented in Table 4. All charcoals were ground and passed through a 2 mm sieve prior to mixing with soil in order to correspond with the particle size of the soil subsamples. Moisture contents were determined by placing charcoals in oven at 105°C for 24 hours.

Table 4. Selected physical and chemical properties of the Leilehua soil and the charcoals used in the incubation experiments.

Soil & Charcoal	Ash	VM	TC	TN	pH_{H2O}	$P^{\dagger}$	K^{+}	Ca^{2+}	Mg^{2+}	Na^{+}	Al^{3+}
	----------		g kg^{-1}	--------		mg kg^{-1}	----------		$cmol_c$ kg^{-1}	----------	
Leilehua soil			42.8	1.2	4.7	2.22	0.09	0.72	0.52	0.29	1.61
34% VM CC	38	340	624								
23% VM CC	31	230	694		7.3						
7.6% VM CC	76	76	823		7.5						
23% VM kiawe	N/A	230	784		6.2						
Raw CC			417								
Raw kiawe			421								

†modified Truog extract; VM=volatile matter and CC=cornocb

The 23% volatile matter content corncob charcoal underwent further treatment in order to determine whether the VM content contained the most bioavailable compounds. We took 1.0 g of charcoal and added 10 ml of 90% acetone in 50 ml centrifuge tubes. Samples were shaken for 30 minutes and passed through a 0.45 micron cellulose filter under vacuum. The acetone extract was collected, diluted five-fold with deionized water, and placed under a nitrogen gas flow to volatilize the acetone. After 2 hours, all the acetone had volatilized and the samples were immediately refrigerated for later use. One hundred ml of deionized water was passed through the remaining, extracted charcoal at 10 ml increments in order to leach any remaining acetone. The extracted charcoals were then collected and placed in an oven at 105°C for 24 hours. Therefore, this treatment permitted us to obtain three fractions of the 23% high VM charcoal:

1. The fresh 23% VM corncob charcoal, which we refer to as the original charcoal. This comprised of the non-treated, **fresh** charcoal.

2. The extracted 23% VM corncob with acetone, which we refer to as the **acetone-extracted** charcoal. This fraction was the material remaining after extraction.

3. The extracted component of the 23% VM corncob with acetone, which we refer to as the **acetone-extractable materials or compouns**. This represents the compounds which were collected as filtrate after the extraction. The acetone was removed via volatilization, leaving only the materials obtained, or extracted, from the charcoal.

In addition to the charcoals collected, we obtained samples of raw corncob husks and kiawe wood prior to carbonization. These materials were also ground to pass through a 2 mm sieve prior to mixing with soil in order to correspond with the particle size of the soil subsamples.

3.2.3 Incubation Study #1

The aim of the first incubation was to determine the effect of VM content on microbial, nitrogen and carbon dynamics in nitrogen-rich and nitrogen-limited systems. The Leilehua soil was passed through a 2 mm sieve prior to sample preparation. The soil was then amended with $Ca(OH)_2$ (3 g kg^{-1}) and $Ca(H_2PO_4)_2 \cdot H_20$ (320 mg P kg^{-1}) to eliminate any effects due to acidity or phosphorus deficiency. Incubations were conducted in triplicate for soil mixtures containing corncob charcoals with either 34% or 7% volatile matter content, with the unamended soil as the control. All soil treatments were carried out with and without nitrogen. Each sampling unit contained 36.6 g (oven-dried equivalent) of soil or soil/charcoal mixture. We added 0.915 g of the 34% and 7% VM charcoals to achieve a rate of 2.5% (weight/weight). Incubations with nitrogen treatment received NH_4NO_3 at a rate of 50 mg N kg^{-1}. Incubations were sampled destructively at days 0, 1, 3, 7, 10, 14, 21, and 28. Over the course of the experiment, the moisture was maintained at 50% gravimetric water content in a laboratory maintained at approximately 25°C.

3.2.4 Incubation Study #2

The aim of the second incubation was to determine the interactive effect of charcoal, VM content and feedstock on microbial and nitrogen dynamics in a two-month incubation. We chose a longer incubation period to examine the persistence of effects during the second

month. The Leilehua soil was prepared in the same manner as in the first incubation. Incubations were conducted for soil mixtures containing corncob charcoals with 23% or 7% VM content, 23% VM kiawe charcoal, the acetone-extracted 23% volatile matter corncob charcoal, the activated charcoal, the raw corncob and raw kiawe materials, in addition to a control, in triplicate. All soil treatments were carried out without nitrogen additions. Descriptions of amendments are provided in Table 4. Each sampling unit contained 25.0 g (oven-dried equivalent) of soil or soil/charcoal mixture, which were placed in 250-ml plastic containers and sealed with paraffin, which received six punctures to facilitate air flow. All amendments were added at a 2.0% total carbon basis (weight/weight). To achieve this rate, 0.720 g of 23% VM corncob charcoal was added; 0.608 g, 7% VM corncob; 0.784 g, kiawe charcoal; 1.120 g of raw corncob; and 1.188 g raw kiawe. Incubations were sampled destructively at weeks 0, 1, 2, 4, 6, and 8. Over the course of the experiment, the moisture was maintained at 50% gravimetric water content in laboratory maintained at approximately 25°C.

After two weeks, soil samples were taken from each treatment for micrographs with a scanning electron microscope (SEM). Samples were first fixed in a 2.5% glutaraldehyde in a 0.1M sodium cacodylate buffer solution for 30 minute, followed by 2 wash changes in 0.1 M sodium cacodylate for 30 and 15 minutes each. Samples were dehydrated in a graded ethanol series (20, 30, 50, 70, 85, and 95%), 2-3 changes of five minutes, and then 100% ethanol for three changes of 10 minutes. Samples underwent critical point drying before being mounted onto SEM aluminum stubs with silver paste. Samples received a sputter coat for 40 seconds. Micrographs were taken of each sample with a Hitachi S-4800 Field Emission Scanning Electron Microscope.

3.2.5 Incubation Study #3

A preliminary survey was performed to estimate the abundance of microbial growth on incubated 23% VM corncob, 7% volatile VM corncob, and 23% VM kiawe charcoals without soil. Subsamples of 1 g (<60 mesh) were taken from each charcoal type and placed in 25 ml plastic containers, in triplicate. Each sample received 0.1-ml of microbial inoculant and 1 ml of a modified Hoagland's solution. The inoculant solution was prepared by using 10 ml of deionized water to 1 g of soil. Two separate solutions were obtained from an alkaline soil and from an acidic soil. Both solutions were shaken vigorously by hand, and then set in a water bath for 24 h at 35°C. The suspensions were shaken and allowed to settle. A final solution was obtained by combining the top 30 ml of each microbial solution. The modified Hoagland stock solution contained 2.5 ml of 1 *M* KNO_3, 2.5 ml of 1 *M* $Ca(NO_3)_2$* 4 H_20, 1 ml of 1 *M* $MgSO_4$* 7 H_2O, 1 ml of 1 *M* NH_4NO_3, and 1 ml of 1 *M* KH_2PO_4. The containers were sealed with paraffin, which received six punctures to facilitate air flow. Charcoal samples were maintained at 150% gravimetric water content at 25°C in the dark for two weeks prior to plating.

The recovery and estimation of microbial populations in the charcoals after two weeks was determined in inoculated petri plates with selected media. One-gram of each charcoal type was transferred in triplicate to a dilution bottle containing 99 ml of sterile water and mixed vigorously for 1 minute. A series of four 10-fold dilutions were obtained by aseptically transferring 1 ml of the suspension into 9 ml of sterile water contained in 30 ml test tubes. One-ml inoculum was transferred into petri plate from the 10^{-3} and 10^{-4} dilution series. Three media solutions were prepared, which were specific to bacteria, fungi, or actinomycetes. Approximately 30 ml of the media solution was added to the petri dishes. The counts were determined after incubating for 48 hours at 28°C with the aid of a colony counter.

After this preliminary experiment, a second study was performed to determine the effect of 23% volatile matter corncob charcoal on fungal growth. Treatments included 23% volatile matter corncob charcoal, acetone-extracted volatile matter corncob, and the extractable material from the 23% volatile matter corncob charcoal after the acetone had been flushed with nitrogen gas (see section explaining charcoal collection). One-g of each charcoal type and all of the extracted materials were transferred to 250 ml flasks, in triplicate. Then, 1 ml of inoculant solution was added. The inoculant solution was prepared by collecting fungal colonies isolated from cultured 23% volatile matter corncob charcoal after an incubation with soil microbes (previously explained). Fungal colonies were suspended in 100 ml of sterilized water containing added mineral nutrients. After the addition of the inoculant solution an additional 100 ml of sterilized water was added. In the case of the extract, approximately 90 ml of sterilized water was added to bring of the final volume to 100 ml. The rates of mineral additions were made to achieve a final concentration of 0.25 g K_2HPO_4 L^{-1}, 0.1 $MgSO_4*7H_20$ L^{-1}, 0.025 NaCl L^{-1}, and 1.44 g KNO_3 L^{-1} for each sampling unit. Flasks were stoppered with foam and sealed with aluminum foil.

Following sample preparation, the samples were incubated at 28°C for two weeks. Samples were mixed by hand daily, and placed on a horizontal shaker for 10 minutes every alternate day. After two weeks, we transferred 1 g of charcoal into a dilution bottle containing 99 ml of sterile water and mixed vigorously for 1 minute. A series of 10-fold dilutions were obtained by aseptically transferring 1 ml of the suspension into 9 ml of sterile water in 30 ml test tubes. One-ml inoculum was transferred into petri plate from the 10^{-4} and 10^{-5} dilution series. Three media solutions were prepared, which were specific to fungi. Approximately 30 ml of the media solution was added to the petri dishes. The counts were determined after incubating for 72 hours at 28°C with the aid of a colony counter.

3.2.6 Laboratory analyses

The volatile matter (VM) content of the charcoal was determined by proximate analysis according to ASTM D1762-84, which involves heating the charcoal in a covered crucible to 950°C for 6 minutes and then determining the weight loss that represents VM content. Charcoal ash content was determined by combustion at 750°C for six hours according to the procedure outlined in ASTM D1762-84 (Antal et al., 2000).

Microbial activity was measured using the fluorescein diacetate hydrolysis method, which is a general estimate of hydrolytic enzyme activity in soil that has shown a strong correlation to microbial activity (Green et al., 2005). This analysis determines the capacity of the soil enzyme to cleave fluorescein diacetate and produce fluorescein, which is a fluorescent molecule suitable for colorimetric quantification. Water extractable organic carbon was determined according to Ghani et al. (2003), which included <0.45 μm soluble organic matter in soil filtrate. Total organic carbon (TOC) was determined using Shimadzu TOC Analyzer Model 5000A. Inorganic N was measured by extracting 5 g soil mixture (oven-dried equivalent) with 25ml 2 *M* KCl in the first incubation, and 5 g soil mixture (oven-dried equivalent) with 50 ml 2 *M* KCl in the second incubation. Ammonium-N and nitrate-N were determined colorimetrically using an EasyChem Discrete Analyzer, following methods by Mulvaney (1996). The pH of the soil was measured at a 1 g/ 1ml deionized water ratio.

3.2.7 Statistical analysis

The effects of time, charcoal type, nitrogen, and charcoal type*time and nitrogen*time interactions were analyzed using Proc Mixed repeated measures with unstructured model (SAS 9.1). Both overall effects and effects by time were analyzed. In the first incubation, three

preplanned contrasts tested for significant differences in enzyme activity, ammonium, nitrate and soluble carbon levels between the 34% VM corncob charcoal and the other 2 treatments, without N additions; and between the 7% VM corncob charcoal and the no-charcoal control, without N additions. A final contrast determined whether there were significant differences between 34% volatile matter charcoal treatment and 34% VM charcoal treatment receiving N. In the second incubation, four preplanned contrasts tested for significant differences between the 23% volatile matter corncob charcoal and the 7% VM corncob charcoal, the acetone-extracted charcoal, 23% VM kiawe charcoal, or the no charcoal control.

In the first incubation, a nonlinear regression was modeled for the release of fluorescein from the 34% volatile matter charcoal treatment, with and without N, using the Gompertz-Lay equation (Lay et al., 1998)

$$F = P \cdot \exp\{-\exp[(R_f \cdot e/P) \cdot (\lambda - t) + 1]\} + d \qquad (2)$$

where F is the cumulative fluorescein production (mg kg^{-1} 3 hr^{-1}), P is the fluorescein production potential (mg kg^{-1} 3 hr^{-1}), R_f is the fluorescein production rate, P is the upper asymptote, and λ is the lag-phase time (days), and d is the fitted y-intercept. The best values of R_f, P, λ, and d were evaluated using the non-linear regression with the "Regression Wizard" in SigmaPlot 8.0 by converging the residual sum of square between the experiment and the estimation to a minimum value. These parameters were evaluated by diagnostic procedures including Student-t, Durbin-Watson, and Kolmogorov-Smirnov tests. The R^2 was reported for each fit indicating whether equation 1 is a suitable model for the fluorescein production data.

In the second experiment, the mineralization rates were estimated for the no-charcoal control, 23% and 7% VM and acetone-extracted corncob charcoals, and 23% volatile matter kiawe corncob. This was accomplished by ranking the replicates for ammonium concentrations

at each sampling period and producing a linear regression through each of the correspondingly ranked replicates for all sampling dates. The first week was excluded due to possible effects due to soil disturbance (Beauchamp et al., 1986). The mineralization rates were analyzed using one-way analysis of variance (ANOVA) with SAS 9.2. Mean separation was performed with Fisher's protected Least Significant Difference (LSD) post hoc procedures at $P<0.05$

In the third experiment, the data fungal counts and FDA activity met the assumptions of normality and homoscedasticity and were analyzed with ANOVA. In case of significant effects, multiple mean comparisons were done using Fisher's LSD. All analyses were performed using SAS 9.2 software. Regression analysis was performed the fluorescein production and the fungal colony forming units using Sigma Plot 10.0.

3.3 Results

3.3.1 Incubation #1

Hydrolytic enzyme activity

The highest hydrolytic enzyme activity was observed in the 34% volatile matter content corncob charcoal with the addition of nitrogen, followed by the 34% volatile matter corncob charcoal without nitrogen (Figure 8 and Table 5). The increase in activity occurred after three days of incubation, the rate of which was approximately two-fold greater when nitrogen was added with the charcoal. After two weeks, the hydrolytic activity had more than doubled in the 34% volatile matter charcoal treatments without nitrogen and increased by four-fold with the addition of nitrogen.

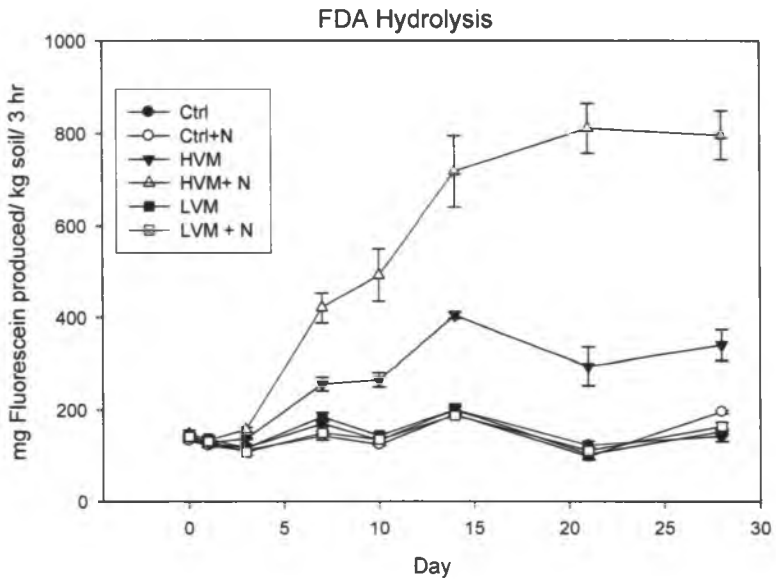


Figure 8. FDA hydrolytic enzyme activity during a one-month incubation of Leilehua soils with additions of different corncob charcoals, with and without nitrogen (N) fertilization. Ctrl=no charcoal control; HVM=34% volatile matter corncob charcoal; and LVM= 7% volatile matter corncob charcoal.

The release of fluorescein in the soils amended with 34% volatile matter charcoal treatments, with and without nitrogen, was fitted with a nonlinear regression model using the Gompertz-Lay equation. This equation estimated the fluorescein production rate (mg kg^{-1} 3 hr^{-1}). This model provided a good fit, with an adjusted R^2 of 0.98 for the 34% volatile matter corncob with nitrogen and 0.78 in the absence of added nitrogen (Figure 9).

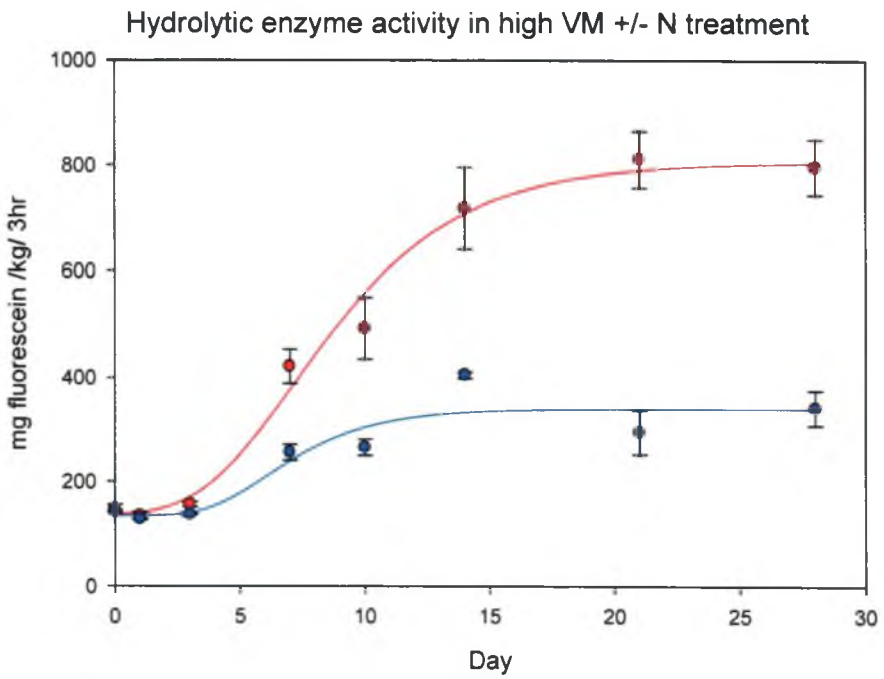


Figure 9. Fitted Gompertz-Lay equation to FDA hydrolytic enzyme activities for Leilehua soils amended with 34% volatile matter corncob charcoal. This pattern follows a typical microbial growth curve. The red line represents the 34% VM corncob charcoal +N; and the blue, 34% VM corncob charcoal without N.

Ammonium and Nitrate

By the end of the first week of incubation, the ammonium content in the soils with 34% volatile matter corncob disappeared entirely (Figure 10a). No ammonium was measured after three and four weeks. In contrast, ammonium concentration in the 7% volatile matter corncob and the no-charcoal control treatments remained constant and similar to each other during most of the experiment. With the addition of nitrogen, ammonium concentration in the 34% volatile matter charcoal treatment decreased significantly relative to the 7% volatile matter charcoal and no-charcoal control by the first week. After three weeks, the 34% volatile matter corncob charcoal treatments had exhausted all of the added ammonium, and contained less ammonium than the no-charcoal control without any nitrogen additions.

With respect to nitrate, there was statistically less extractable nitrate in the 34% volatile matter charcoal treatments in comparison to the 7% volatile matter charcoal and no-charcoal control treatments after the first week, but no significant differences between the extractable nitrate in the 7% volatile matter corncob charcoal and no-charcoal control treatments for the entire incubation (Table 5). With the addition of nitrogen, there was significantly less extractable nitrate in both the 34% and 7% volatile matter corncob charcoal treatments relative to the no-charcoal control after seven days, though the 7% volatile matter corncob treatments consistently contained relatively more nitrate than the 34% volatile matter corncob charcoal (Figure 10b).

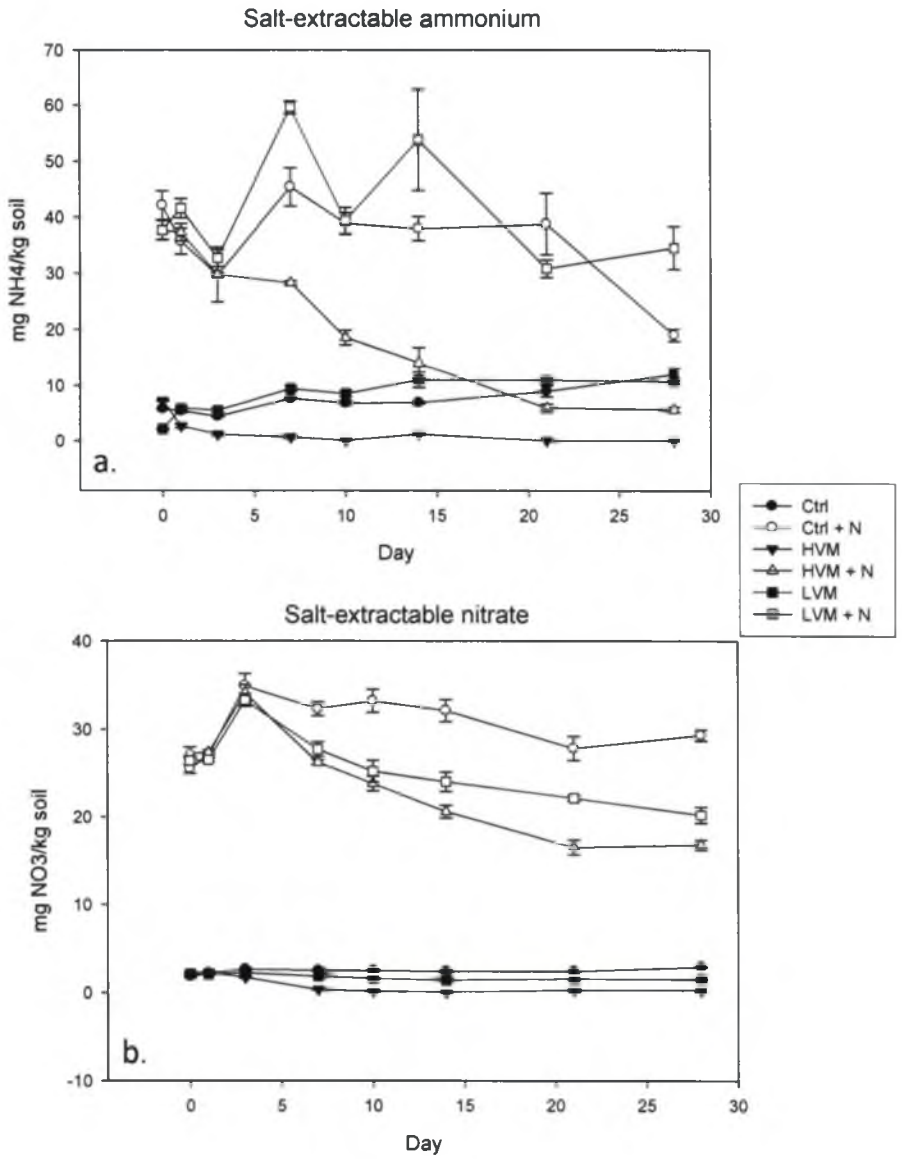


Figure 10. Fluctuations in soil ammonium (a) and nitrate (b) upon the additions of charcoals, with and without nitrogen (N), in time. Ctrl=no charcoal control; HVM=34% volatile matter corncob charcoal; and LVM= 7% volatile matter corncob charcoal.

Water extractable carbon

The soils receiving the 34% VM corncob charcoal had significantly greater amounts of water extractable carbon than the 7% volatile matter charcoal treatments, and both charcoal treatments contained more extractable carbon than the soil alone (Table 5). Changes in the water extractable carbon levels in the 7% volatile matter corncob treatments largely mirrored those of the no-charcoal control throughout the entire incubation. In contrast, the 34% volatile matter corncob charcoal treatments showed a two-fold loss of water extractable carbon relative to both the no charcoal control and the 7% volatile matter corncob charcoal treatments within two weeks (Figure 11).

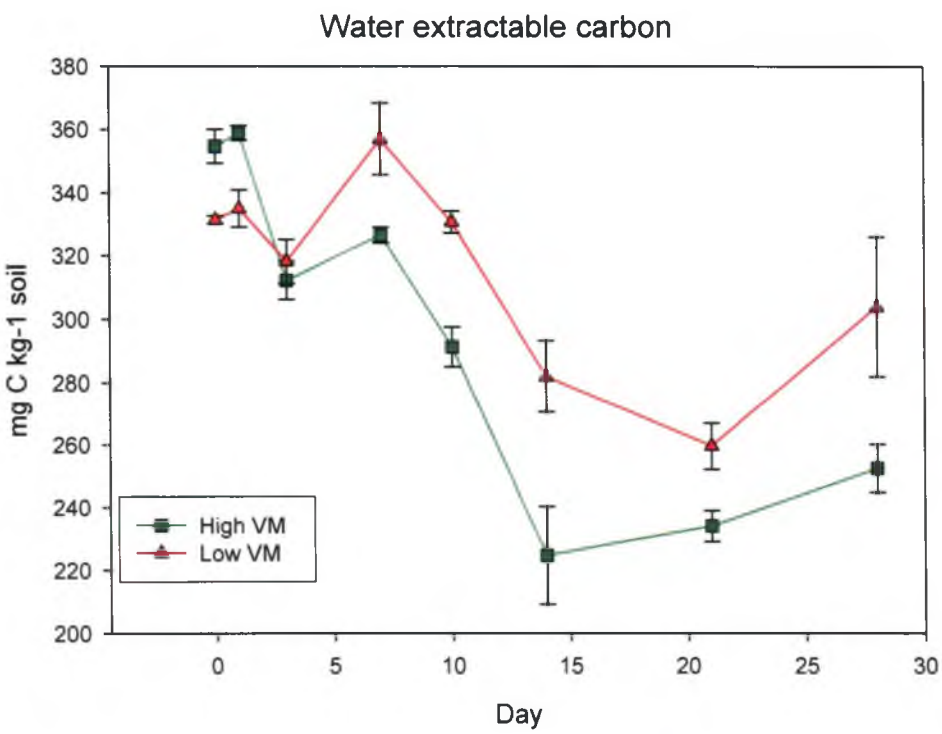


Figure 11. Water extractable organic carbon fluctuations upon the additions of 34% volatile matter corncob charcoal (High VM) and 7% volatile matter corncob charcoal (Low VM) with time.

Table 5. Statistical results for overall effects and preplanned contrast in Incubation 1

FDA hydrolytic enzyme activity				
Overall Effect		Preplanned contrasts		
Effect	p-value	Treatments	p-value	Effect by time
Treatment	<0.0001	HVM > LVM and Control	<0.0001	Day 3 and remainder
Time	<0.0001	LVM > Control	0.7457	Entire incubation
Nitrogen Addition	0.2461	HVM+N > HVM	<0.0001	Day 3 and remainder
Treatment* Time	<0.0001			
Nitrogen addition*Time	<0.0001			
Ammonium				
Overall Effect		Preplanned contrasts		
	p-value	Treatments	p-value	Effect by time
Treatment	<0.0001	HVM < LVM and Control	<0.0001	Day 7, 10, 21, 28
Time	<0.0001	LVM < Control	0.1426	Entire incubation
Nitrogen Addition	<0.0001	HVM+N > HVM	<0.0001	Entire incubation
Treatment* Time	<0.0001			
Nitrogen addition*Time	<0.0001			
Nitrate				
Overall Effect		Preplanned contrasts		
	p-value	Treatments	p-value	Effect by time
Treatment	<0.0001	HVM > LVM and Control	0.0268	Days 7, 21, 28
Time	<0.0001	LVM > Control	0.2803	Entire incubation
Nitrogen Addition	<0.0001	HVM+N > HVM	<0.0001	Entire incubation
Treatment* Time	<0.0001			
Nitrogen addition*Time	<0.0001			
Water extractable carbon				
Overall effect		Preplanned contrasts		
	p-value	Treatments	p-value	Effect by time
Treatment	<0.0001	HVM < LVM and Control	0.0328	Day 3, 7, 10, 21, 28
Time	<0.0001	LVM > Control	<0.0001	Entire incubation
Treatment* Time	0.0009	HVM+N < HVM	0.2367	Entire incubation

3.3.2 Incubation #2

Hydrolytic enzyme activity

In the first incubation, we focused on the effect of charcoal VM content and nitrogen availability on biological processes. In the second incubation, we added another variable of feedstock to determine whether charcoals with similar VM contents, but different feedstocks exhibited similar behavior. The soil hydrolytic enzyme activity was significantly increased upon the addition of the corncob husk feedstock, followed by the raw kiawe wood. The 23% volatile matter corncob charcoal was only charcoal that significantly enhanced enzyme activity (Table 6). In contrast, the hydrolytic enzyme activity was not significantly enhanced relative to the no-charcoal control upon the additions of 23% volatile matter kiawe charcoal and 7% volatile matter corncob charcoal (Figure 12). Furthermore, the hydrolytic enzyme activity in the soils with added acetone-extracted 23% volatile matter corncob charcoal was significantly less than the original 23% volatile matter corncob charcoal during the first month of incubation, but the activity increased to that of the original charcoal during the second month of incubation (Figure 13). The SEM micrographs provided visual insight into the surface structure of charcoal, its interactions with soil particles, and the presence of fungal growth after two weeks of incubations. In general, we found prolific biological activity in the treatments receiving the raw feedstock materials (Figure 14C-F) in comparison to the soil alone or receiving activated charcoal (Figure 14A and B). We also provide evidence of fungal hyphae on the surface of the 23% VM corncob charcoal (Figure 14G-K), including the fresh and the extracted treatments. We did not capture the presence of fungal growth on the kiawe and 7% VM corncob charcoals (Figure14L-O).

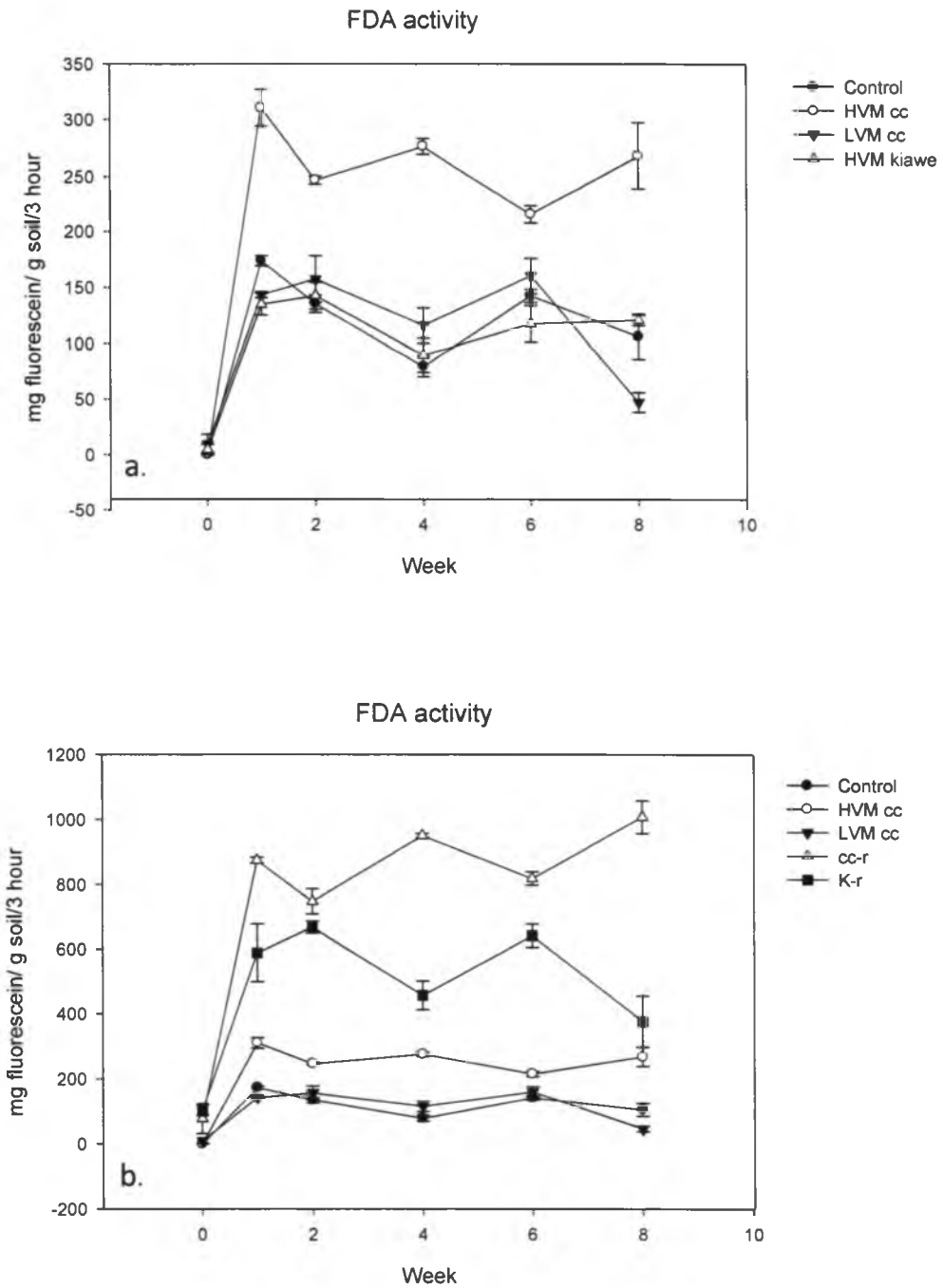


Figure 12. Effect of high VM corncob and kiawe charcoals and low VM corncob charcoal (a) and raw corncob and kiawe feedstocks (b) on FDA hydrolytic enzyme activity in the Leilehua soil during a 2-month incubation. Ctrl=no charcoal control; HVM cc = 23% volatile matter corncob charcoal; LVM cc= 7% volatile matter corncob charcoal, HVM kiawe = 23% volatile matter kiawe charcoal; cc-r= raw corncob husks; K-r = raw kiawe wood.

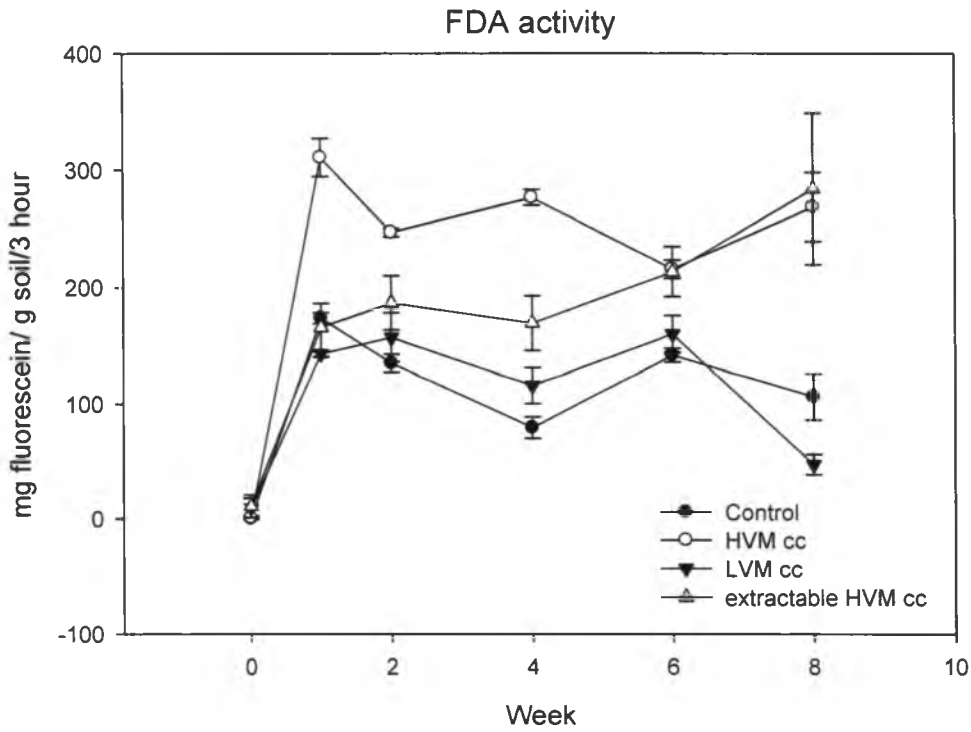


Figure 13. Effect of extracting 23% volatile matter corncob with acetone prior to addition to soil (extractable HVM cc) on FDA activity. The effect extracted charcoal did not widely differ from the 7% volatile matter corncob charcoal (LVM cc) during first two weeks of incubation, but its FDA activity reached that of the 23% volatile matter corncob charcoal (HVM cc) by 6 weeks.

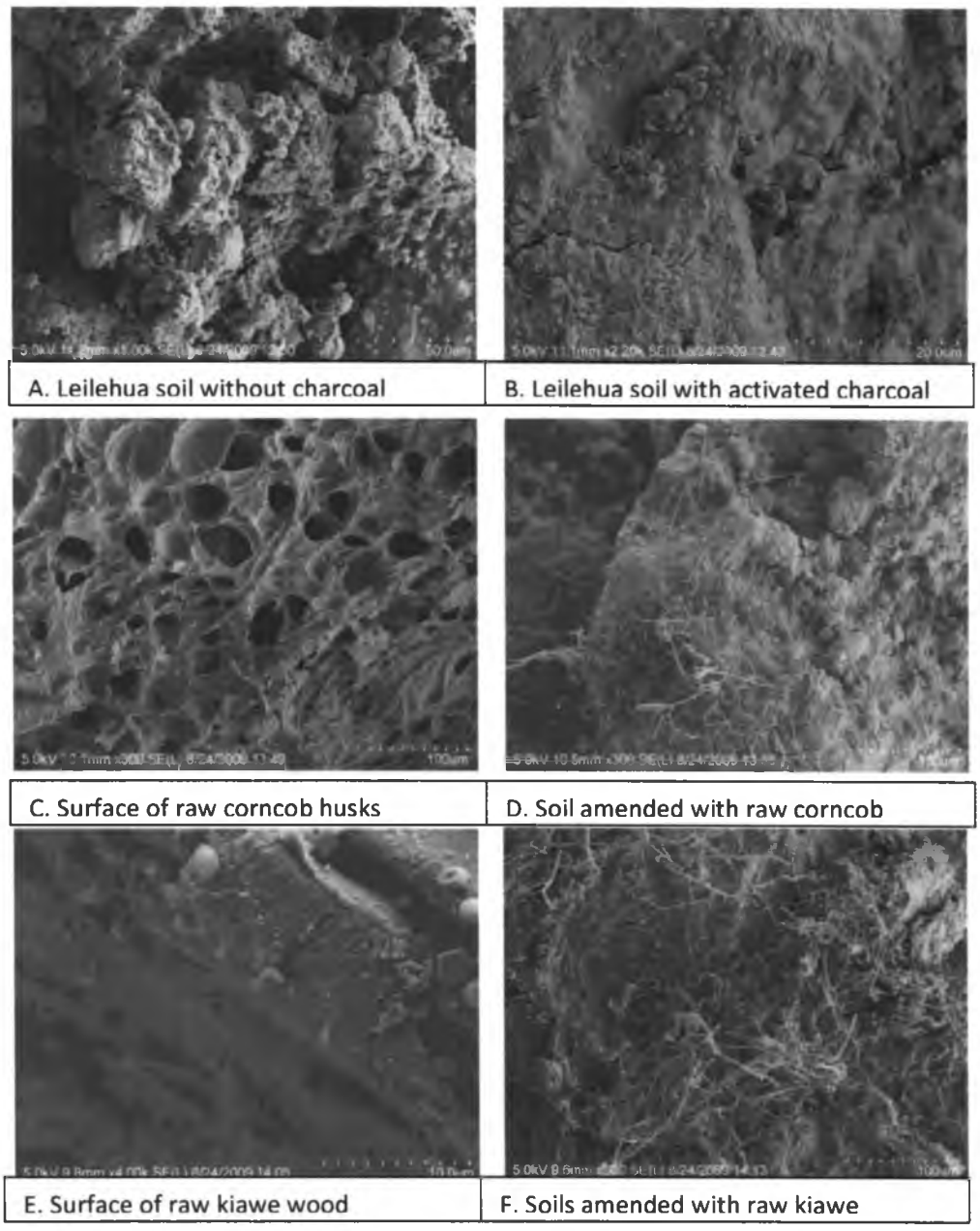
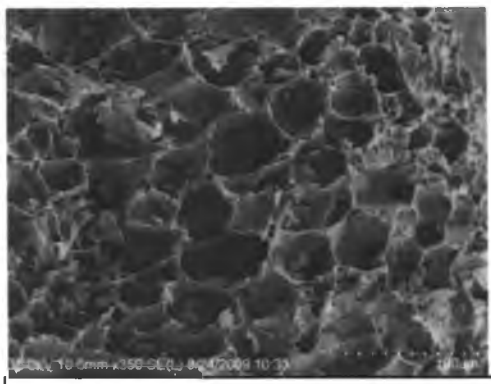
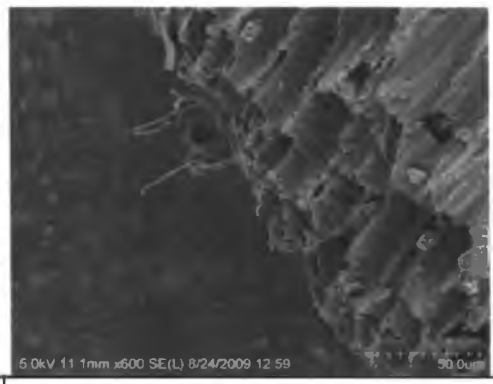


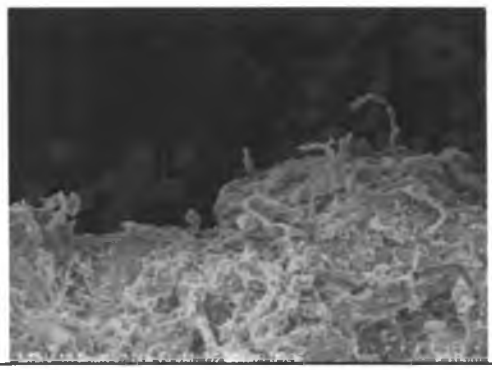
Figure 14 A-F. SEM micrographs of soils with various charcoals and amendments.



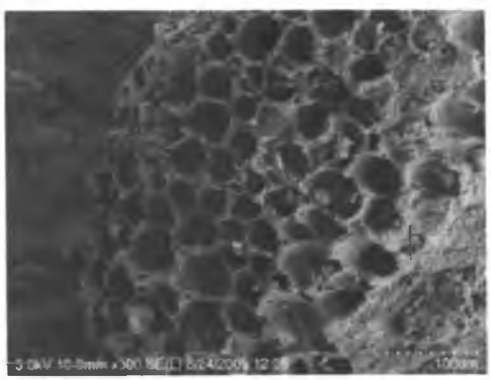
G. Surface of 23% VM corncob charcoal



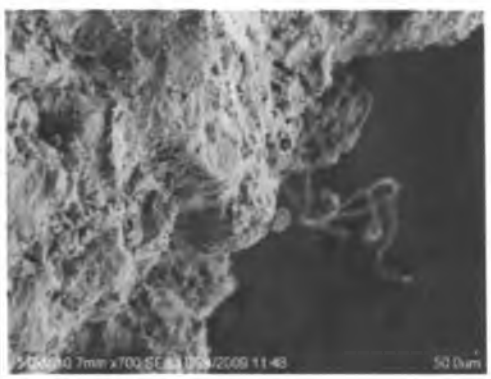
H. Possible fungal growth on 23% VM



I. Soil amended with 23% VM corncob charcoal

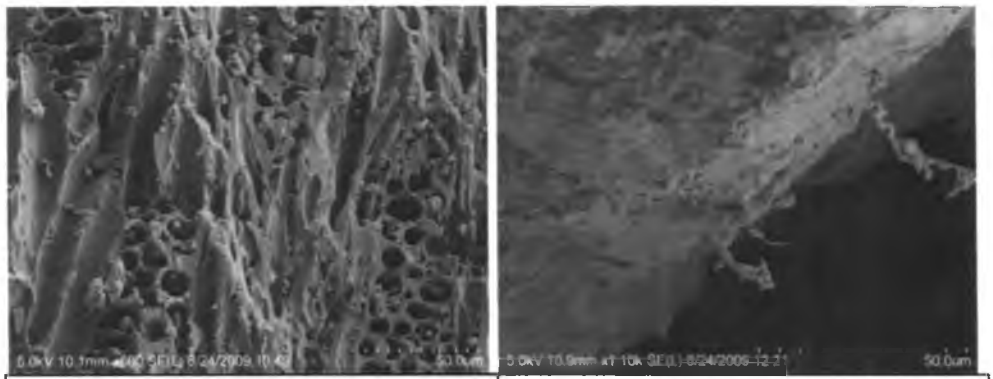


J. Surface of extracted corncob char

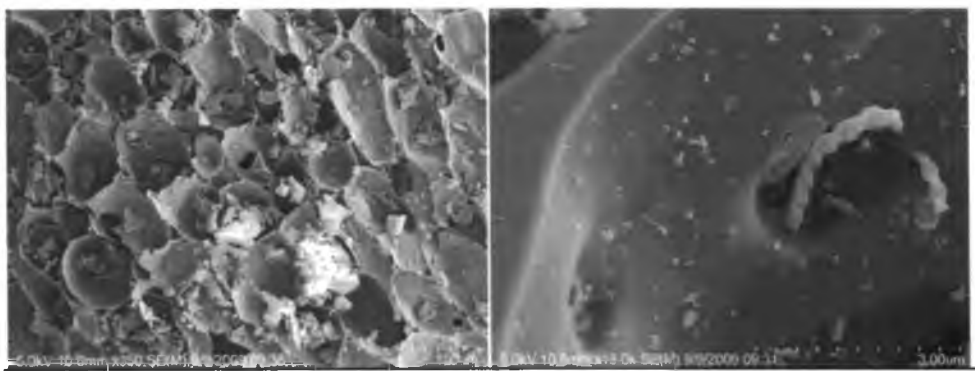


K. Soil with extracted corncob charcoal

Figure 14 G-K. SEM micrographs of soils with various charcoals and amendments.



L. Surface of23% VM kiawe charcoal | M. Soil amended with kiawe charcoal

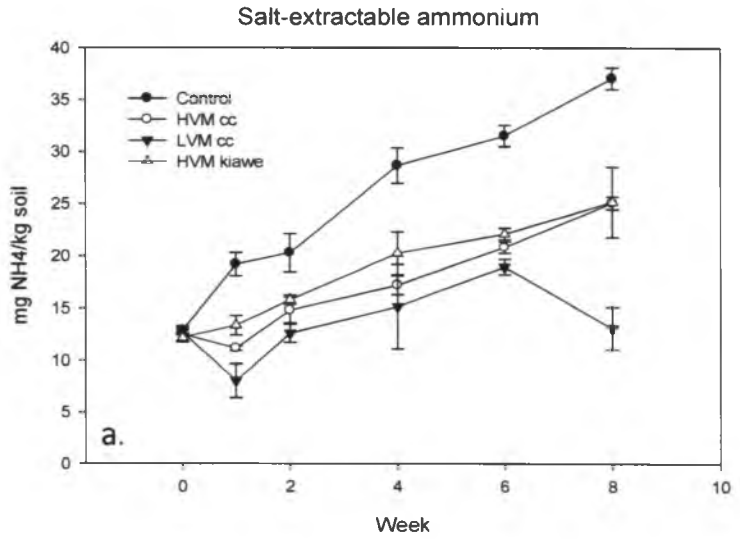


N. Surface of 7% VM corncob char | O. Surface of 7% VM corncob charcoal

Figure 14 L-O. SEM micrographs of soils with various charcoals and amendments.

Ammonium and nitrate

The no-charcoal soil control showed nitrogen greater mineralization during the two months in comparison to Incubation #1. Additions of the 23% and 7% volatile matter corncob and 23% volatile matter kiawe charcoal resulted in significantly less ammonium than the control (Table 6), but all showed a net mineralization of nitrogen throughout the two month incubation with the exception of 7% volatile matter corncob at week 8. The additions of the raw feedstock materials both resulted in the exhaustion of ammonium after one week. The acetone-extracted 23% volatile matter charcoal treatment showed a similar trend during the first 2 weeks of the incubation as the original 23% volatile matter charcoal, but showed a significant decline in ammonium after four weeks (Figure 15).



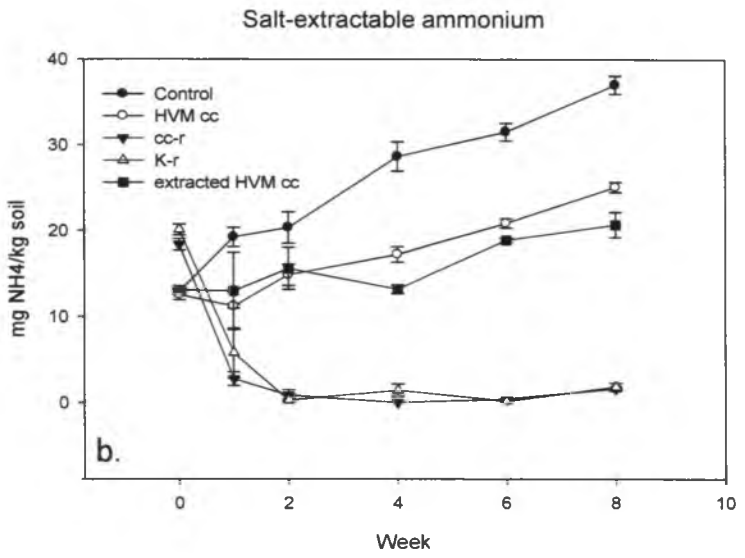


Figure 15. Effect of high VM corncob and kiawe charcoals and low VM corncob charcoal (a) and raw corncob and kiawe feedstocks (b) on ammonium levels in the Leilehua soil during a 2-month incubation. Ctrl=no charcoal control; HVM cc = 23% volatile matter corncob charcoal; LVM cc= 7% volatile matter corncob charcoal, HVM kiawe = 23% volatile matter kiawe charcoal; cc-r= raw corncob husks; K-r = raw kiawe wood.

The rate of ammonium production (Figure 16) was significantly greatest in the no-charcoal control, which was approximately 0.36 mg NH_4 day^{-1}. The mineralization rates for these charcoal treatments were statistically the same. The 23% volatile matter corncob charcoal had the production of 0.26 mg NH_4 day^{-1}; 23% volatile matter kiawe charcoal, with 0.23 mg NH_4 day^{-1}; and acetone-extracted 23% volatile matter corncob charcoal, 0.24 mg NH_4 day^{-1}. The 7% volatile matter corncob charcoal had a weak mineralization pattern due to the decline in ammonium at two months. With the inclusion of the final week, the regression of mineralization rate was not linear and estimated at 0.12 mg NH_4 day^{-1}, and was significantly lower than all other charcoal types. However, with the exclusion of the final week, the mineralization rate was 0.29 mg NH_4 day^{-1}, which was statistically the same as the other charcoal treatments. These data indicate that the additions of charcoal, despite its VM content, reduced the mineralization of nitrogen in soil.

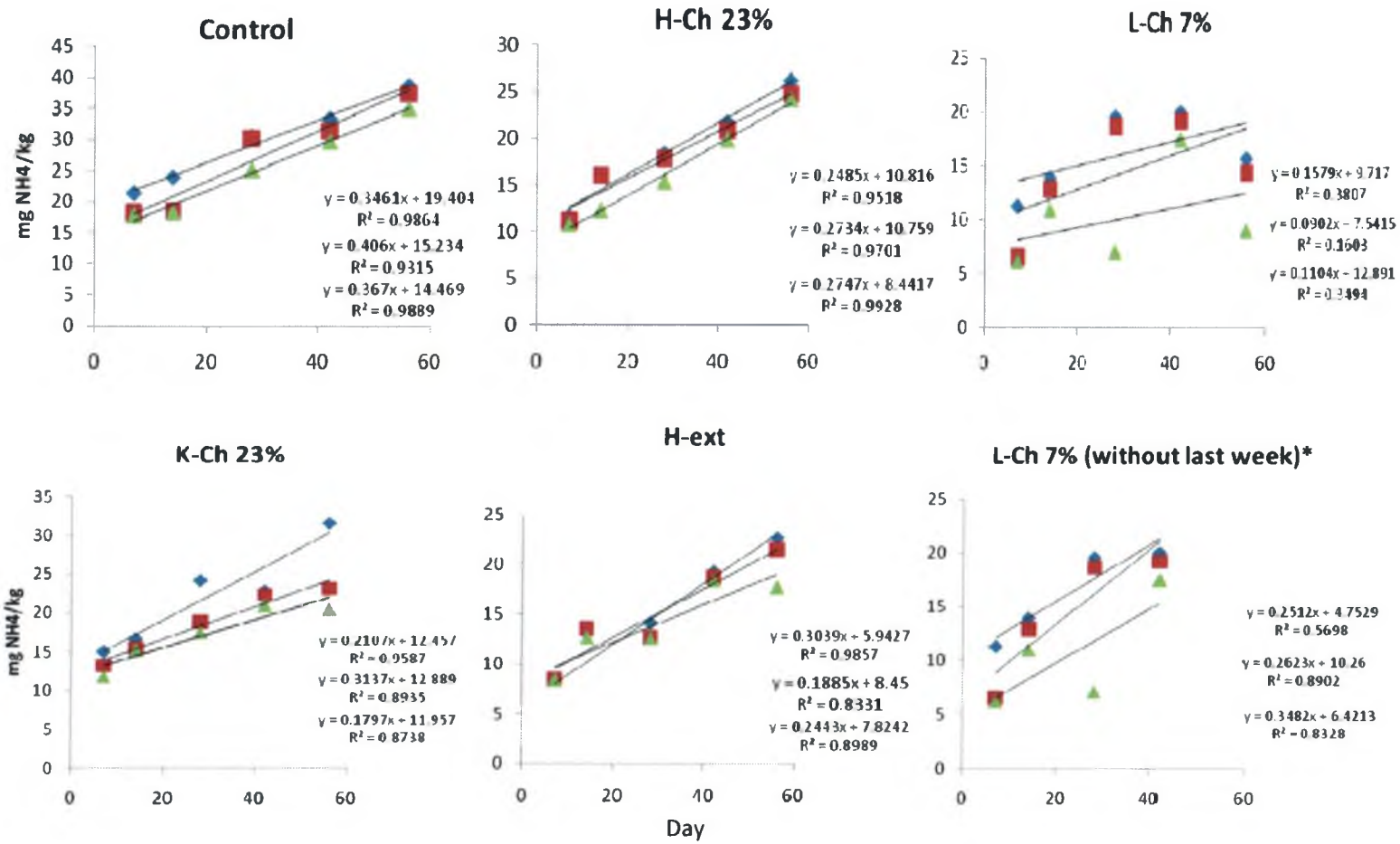


Figure 16. Mineralization rates for soils receiving charcoal treatments. Mineralization data during first weeks was ignored. H=23% VM corncob; L=7% VM corncob; K=23% VM kiawe; Ch=Charcoal; ext=23% VM charcoal extracted with acetone. *The last week was ignored due to non-linear behavior of ammonium. Different symbols represent the triplicated samples.

The nitrate levels were significantly greatest in the 7% volatile matter corncob charcoal treatments by the fourth week of the incubation (Figure 17). The 23% volatile matter corncob and kiawe charcoals did not show significant differences from the no-charcoal control until six and eight weeks of incubation, in which the no-charcoal control contained greater amounts of nitrate. The addition of both raw feedstock materials resulted in significantly less nitrate, although these treatments followed the same general trends as the other treatments during the course of the incubation. There were no differences between the amount of nitrate in the original 23% volatile matter corncob and its acetone-extracted counterpart.

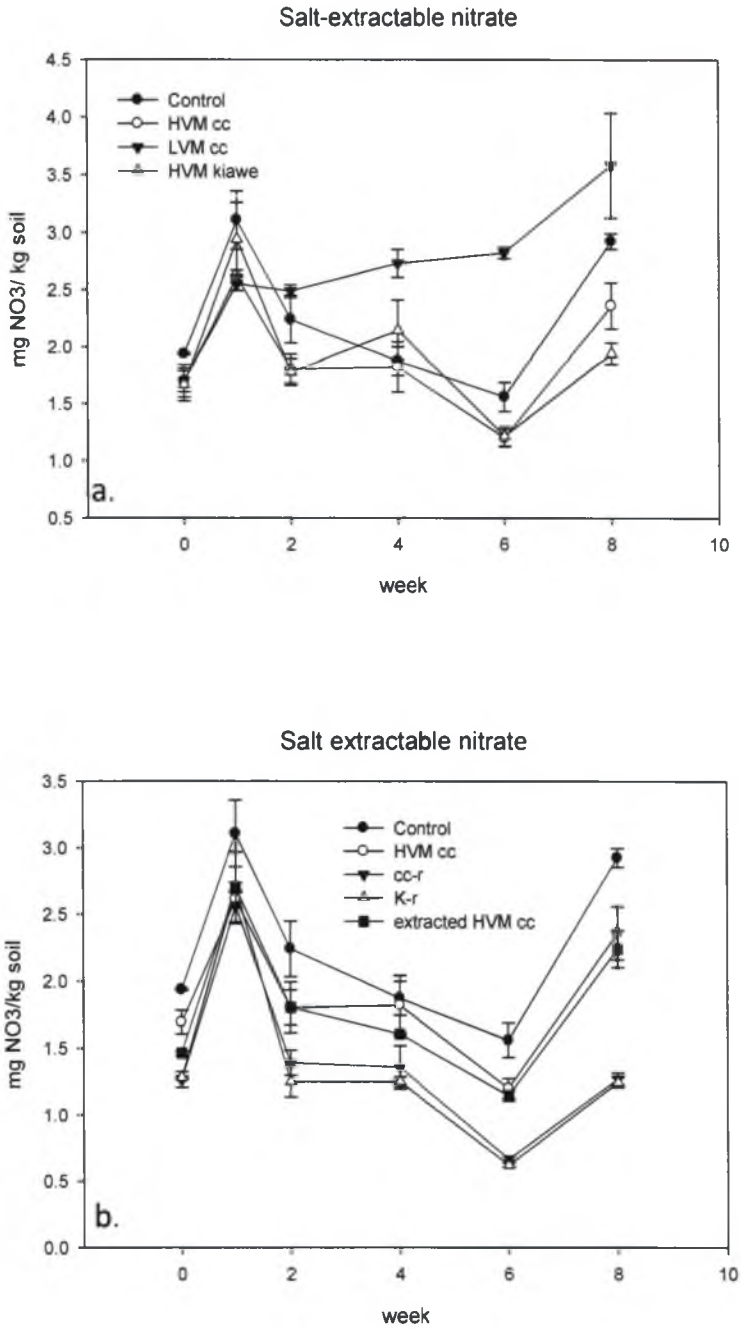


Figure 17. Effect of high VM corncob and kiawe charcoals and low VM corncob charcoal (a) and raw corncob and kiawe feedstocks (b) on nitrate levels in the Leilehua soil during a 2-month incubation. Ctrl=no charcoal control; HVM cc = 23% volatile matter corncob charcoal; LVM cc= 7% volatile matter corncob charcoal, HVM kiawe = 23% volatile matter kiawe charcoal; cc-r= raw corncob husks; K-r = raw kiawe wood.

pH

The pH of the no-charcoal controls ranged from approximately 6.05 to 6.25. The only treatments with a strong effect on soil pH were those with additions of raw feedstock materials. The raw corncob husk treatments caused a decline in pH to 5.0 after two weeks, which subsequently increased to 5.3 after two months. The addition of raw kiawe wood reduced pH from 6.0 to 5.6 within the two month incubation (data not shown).

Table 6. Statistical results for overall effects and preplanned contrasts in Incubation 2

FDA hydrolytic enzyme activity				
Overall Effect		Effect by time		
Effect	p-value	Treatment	P-value	Time
Treatment	<0.0001	23% VM corncob char> Control	<0.0001	Week 1 and remainder
Time	<0.0001	23% VM corncob >7% VM corncob	<0.0001	Week 1 and remainder
Treatment*time	<0.0001	23% VM corncob > 23% VM kiawe	<0.0001	Week 1 and remainder
		23% VM corncob> acetone extracted	0.0007	Week 1 - 4
Ammonium				
Overall Effect		Effect by time		
Effect	p-value	Treatment	P-value	Time
Treatment	<0.0001	23% VM corncob char< Control	<0.0001	Week 1 and remainder
Time	<0.0001	23% VM corncob <7% VM corncob	<0.0001	Entire incubation
Treatment*time	<0.0001	23% VM corncob > 23% VM kiawe	0.1223	Entire incubation
		23% VM corncob< acetone extracted	0.0045	Entire incubation
Nitrate				
Overall Effect		Effect by time		
Effect	p-value	Treatment	P-value	Time
Treatment	<0.0001	23% VM corncob char< Control	<0.0001	Week 0, 1, 2, 6
Time	<0.0001	23% VM corncob <7% VM corncob	<0.0001	Week 2 and remainder
Treatment*time	<0.0001	23% VM corncob > 23% VM kiawe	0.7060	Entire incubation
		23% VM corncob< acetone extracted	0.3101	Week 1 - 8

3.3.3 Incubation #3

The goal of the third incubation was to determine whether the fraction of high VM corncob which was extractable with acetone stimulated fungal growth and activity. The preliminary microbial counts indicated that 23% VM corncob charcoal supported the most fungi, bacteria, and actinomycetes (Table 7). The 23% VM kiawe charcoal contained approximately the same amount of fungi as the corncob charcoal with the same VM content, but lesser amounts of bacteria and actinomycetes. The low VM corncob charcoal supported the least fungi, but greater amounts of actinomycetes and bacteria than the kiawe charcoal.

Table 7. Enumeration of microbial groups isolated from charcoals.

	Fungi	Actinomycetes	Bacteria
23% VM CC	$3.8*10^6$	$5.2*10^7$	$7.2*10^7$
23% VM kiawe	$3.8*10^6$	$5.7*10^6$	$5.2*10^6$
7% VM CC	$3.0*10^4$	$1.2*10^7$	$1.3*10^7$

VM=volatile matter; CC=corncob

In the follow-up study, we tested the effect of various components of the 23% volatile matter corncob charcoal on fungal growth and activity. We found that the fungal counts and hydrolytic enzyme activity were greatest in the acetone-extractable materials from the 23% volatile matter corncob charcoal (Figure 18). This was followed by the fresh 23% volatile matter corncob charcoal and the acetone-extracted charcoal. The fungal activity, but not counts, was significantly less in the acetone-extracted charcoal than the original. The no-charcoal control had significantly lower fungal growth and activity than all the charcoal treatments (Figure 18). A regression analysis shows that there is a strong relationship between the hydrolytic enzyme activity and fungal colony forming units, which had a R^2 of 0.76 (Figure 19).

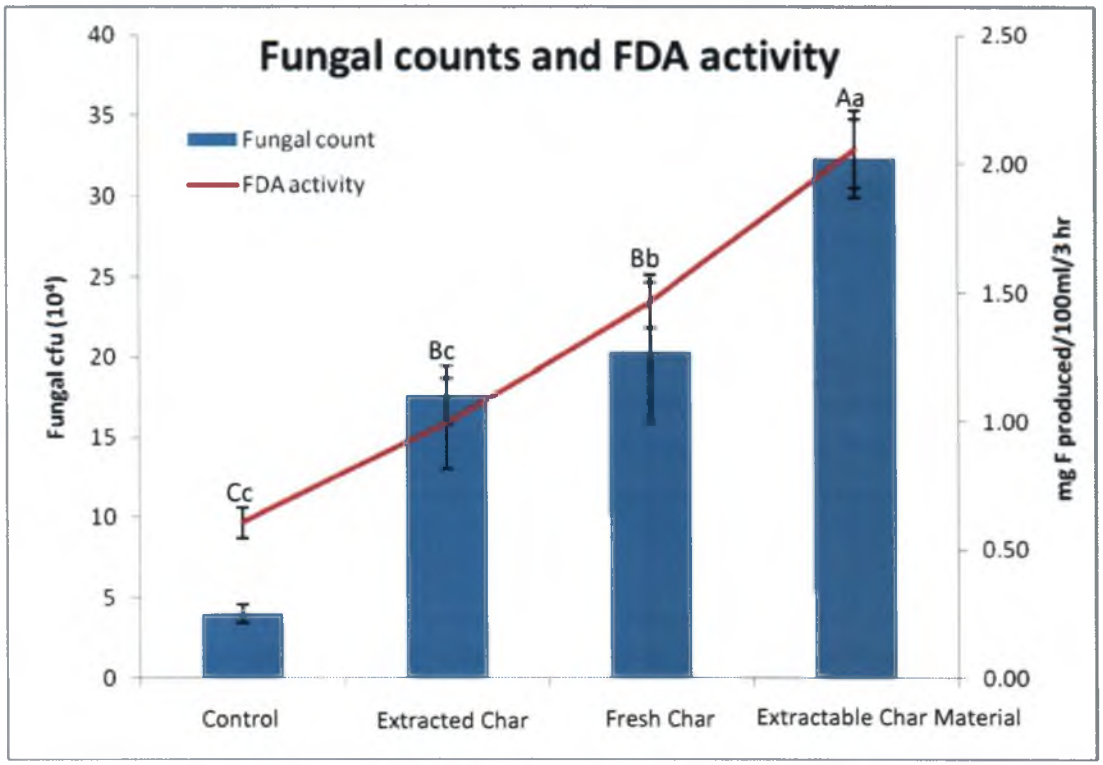


Figure 18. Fungal counts and FDA activity for the control, acetone extracted high volatile matter charcoal, high volatile matter charcoal, and acetone extractable matter from the charcoal. The error bars represent standard error of the means (n=3). Fungal counts denoted by the same uppercase letter.

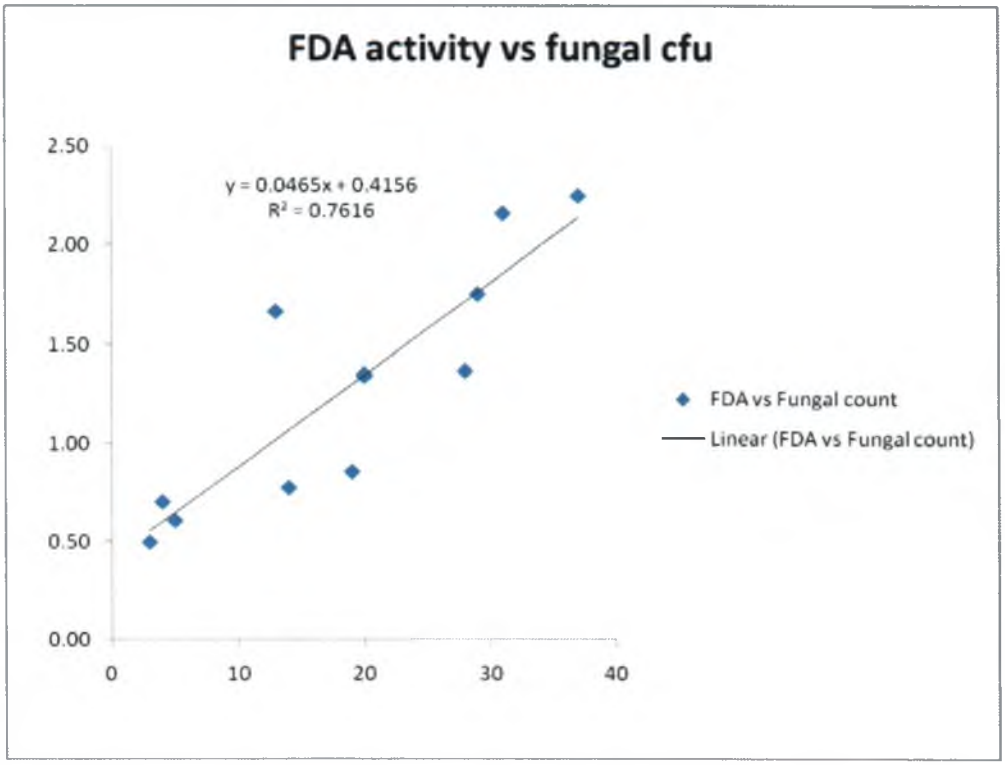


Figure 19. Relationship between FDA activity and fungal colony forming units

3.4 Discussion

3.4.1 Microbial activity

Our results show that charcoals differ in their labile carbon pools according to their VM content or degree of thermal alteration, in agreement with previous studies (Hamer et al., 2004; Baldock and Smernik, 2002; Czimczik and Masiello, 2007; Bruun, 2008). Our results clearly show the differential effect of a spectrum of charred corncob materials on soil microbial activity. The addition of raw corncob husk materials enhanced microbial activity by almost 7-fold and the 34% VM corncob charcoal resulted in a 2.5-fold increase in activity. This was followed by 23% VM corncob charcoal, which also doubled the microbial activity. In comparison, the 7% VM corncob charcoal had no effect on soil microbial activity. Thus, the effect of charcoal on microbial activity was inversely related to its degree of carbonization. Our results agree with Baldock and Smernik (2002), who found that the conversion of alkyl carbon to aryl carbon during pyrolysis corresponded with a reduction in carbon mineralization. In a bioavailability study, these authors showed that charring sapwood to temperatures greater than 200°C resulted in a 10-fold decrease in the mineralization of the sapwood by microbial inoculum.

In our first incubation study, we provided four indirect lines of evidence showing the bioavailability of the high VM corncob charcoal. First, the enhancement of microbial activity by the high VM corncob charcoal appeared to be limited by nitrogen, rather than carbon as evidenced by the 4-fold increase in microbial activity when nitrogen was applied in combination with the 34% VM corncob. Gundale and De Luca (2007) also showed that the addition of a labile nitrogen substrate relieved nitrogen limitations and enhanced carbon dioxide respiration in soils amended with charcoal produced at 350°C (low temperature). Secondly, we observed the exhaustion in the soil nitrogen receiving 34% VM corncob indicating strong nitrogen

immobilization, unlike the low VM charcoal. Similarly, Gundale and De Luca (2007) showed that the addition of low temperature charcoal, which presumably contained relatively high VM content, strongly immobilized soil nitrogen. Thirdly, the stimulation of hydrolytic enzyme activity follows a typical microbial growth pattern upon the addition of 34% volatile matter corncob (Lay et al., 1998). Finally, the SEM micrographs provide evidence of colonization of fungi on 23% VM charcoal. Hockaday et al. (2007) also reported extensive fungal hyphae development on the surface of circa 100-year old charcoal. This study suggested that fungi are instrumental in the degradation of charcoal and formation of oxidized PAHs. These data combined with the FTIR, NMR, GC-MS results reported in Chapter 2 suggest that high VM corncob charcoal has a labile and bioavailable C pool different in composition and bioavailability from that in the low VM charcoal.

In the second and third incubations, we provide more compelling evidence that the high VM corncob charcoal contains bioavailable carbon compounds which stimulate microbial growth. For these incubations, we fractionated the 23% VM corncob charcoal with acetone to obtain the readily extractable (e.g. soluble) components of the charcoal, as well as an extracted charcoal (e.g. stripped of soluble materials). When we added the extracted charcoal to soil, we showed that the charcoal did not enhance soil microbial activity during the first month of incubation. This was in major contrast to the fresh charcoal, which stimulated activity within the first week. By the sixth week of incubation, their effect on soil microbial activities was the same. These data provide two important pieces of information. First, the most labile compounds in the high VM charcoal can be removed with acetone. Secondly, we showed that other compounds remained after an extraction of charcoal with acetone, which are relatively more recalcitrant and not readily available within the first month, but with increasing time serve as a substrate for soil microorganisms.

In the third incubation, we showed that the acetone-extractable fraction of the 23% VM corncob charcoal directly supported fungal growth and activity. In this experiment, we obtained the compounds that were removed from the charcoal during the extraction. We determined that this fraction supported significantly greater fungal growth and activity. Steiner et al. (2008) also fractionated charcoal by condensing the tarry vapors, or pyroligneous acid, which consisted of acids, alcohols, aldehydes, ketones, and sugars. These researchers found the pyroligneous acid strongly enhanced the microbial growth within hours of its addition, particularly in combination with glucose. High VM charcoals are known to contain recondensed tarry vapors on the surface of the charcoal, in addition to partially carbonized compounds (Meszaros et al., 2007), which could be dissolved by acetone. In our study, the extractable materials supported greater fungal growth and activity than the extracted charcoal (e.g. stripped of soluble compounds) and even the fresh charcoal. Though the fresh charcoal supported significantly greater fungal activity than the extracted charcoal, there were not statistical differences in their fungal counts. Therefore, we showed that bioavailable compounds are removed with acetone, but some materials remain albeit more recalcitrant. This observation supports the findings in the second incubation which showed an enhancement in microbial activity after 6 weeks of the addition of the extracted charcoal. Our findings suggest that acetone is an appropriate solvent to extract the most labile compounds, but is not a strong enough to extract the more recalcitrant compounds which become available after the first month. We propose that a fractionation approach be used to sequentially remove compounds with differing solubility characteristics.

Despite containing a high VM content, the kiawe charcoal did not stimulate microbial activity. Correspondingly, we did not detect any compounds in its acetone-extractable fraction (Chapter 2), which provided a bioavailable pool for the high VM corncob charcoal. Interestingly,

the raw kiawe wood resulted in half the soil microbial activity as the corncob feedstock. Thus, kiawe wood and its charcoal derivative were inherently less degradable than the corncob feedstock and charcoal. In agreement with our findings, Hamer et al. (2004) observed differences between the mineralization of carbon of wood and corncob charcoals. They determined that wood charcoal derived at 250°C had approximately three times less carbon mineralization during a two-month incubation than corncob charcoal produced at the same temperature. We hypothesize that there are fundamental differences between the molecular composition of kiawe and corncob, which influences it's the bioavailability of their raw and charcoal materials.

3.4.2 Nitrogen

Our previous work has shown that VM content in charcoal strongly reduces nitrogen availability in soil (Deenik et al., 2010). Other researchers have also observed the immobilization of nitrogen in soils receiving low-temperature (Gundale and De Luca, 2007) and high VM charcoals (Rondon et al., 2007). However, the effect of VM content on nitrogen dynamics appears somewhat convoluted in the present study. During the first incubation, the addition of 34% VM corncob charcoal resulted in a complete exhaustion of soil ammonium and nitrate. The disappearance of ammonium and nitrate combined with the increase in microbial activity suggests that the presence of bioavailable carbon in the high VM char caused nitrogen immobilization, in agreement with observations in a two week incubation by Deenik et al. (2010). Even when nitrogen was added along with the higher VM corncob charcoal, it was rapidly consumed during the one-month incubation providing further evidence that immobilization processes were at work. Consistent with our expectations, the 7% VM charcoal showed no significant effect on N dynamics during the 4-week incubation. However, unlike the first incubation, the higher VM corncob charcoal (23%) used in the second incubation did not

result in the complete immobilization of nitrogen. Though there appeared to be a significant reduction in the mineralization rate relative to the control, the effect was not as dramatic as the 34% VM charcoal. This might be due to several factors. First, the VM of corncob charcoal was 10% less in the second incubation, which suggests a lesser bioavailability. Second, the initial soil ammonium levels were more than twice as high, and thus nitrogen was not as limited. Thirdly, the soil alone control mineralized ammonium more rapidly than in the first incubation. Though the soil used in this incubation was the same type of soil used in the one-month incubation, it was obtained from a different batch at a later time.

The influence of the 7% VM corncob on soil nitrogen dynamics in the second incubation also diverges from the first. In the second incubation, there was less extractable ammonium than the no-charcoal control and even the higher volatile matter treatments. This persisted throughout the entire incubation. The reduction in ammonium due to the low VM charcoal was puzzling. At first we speculated that the lower ammonium levels were due to adsorption by the low VM charcoal. However, a quick laboratory test showed that the ammonium sorption was not significant in soil amended with 7% volatile matter corncob charcoal at a 2.5% rate (data not shown). A better explanation is that 7% VM corncob charcoal enhanced the nitrification rate in the second incubation, as the nitrate data shows a significant increase in net nitrification.

Despite having no effect on soil microbial activity, the 23% VM kiawe also reduced soil nitrogen, similar to the other charcoals. Our preliminary enumeration of charcoal microorganisms showed that kiawe charcoal supported a high fungal biomass, but much lower bacteria and actinomycetes as compared to the other charcoals. We speculate that the kiawe might be supporting microbial populations that are not functioning in the soil, but the mechanism is unknown.

The goal of our research was to relate VM content in charcoal to nitrogen dynamics. The VM content is a measurable property of charcoal and inversely related to production temperature. Though not a quantifiable charcoal property, previous researchers have described charcoal in terms of production temperature. In general, our results agree with other studies investigating the effects of low and high temperature charcoal (350°C) on mineralization and nitrification. We found that the high VM charcoals resulted in net immobilization relative to the soils alone, which was not accompanied by an increase in net nitrification. Gundale and DeLuca (2007) reported that the addition of the low-temperature charcoal without glycine, a readily available organic N source, resulted in declined nitrification and mineralization. In comparison, De Luca et al (2006) showed that nitrification was enhanced with the addition of wildfire charcoal, which presumably might be higher temperature charcoals (DeLuca et al., 2006). These authors attribute the enhancement in nitrification to increased sorption of nitrification-inhibiting molecules, such as phenols. While this explanation seems unlikely in our case given the absence of phenols, the lower VM charcoal could provide a habitat for nitrifying bacteria. The enumeration of bacteria in the preliminary plating study showed that the low VM charcoal supported a greater bacterial population than the high VM kiawe charcoal (whereas we isolated magnitudes less fungi compared to both high VM charcoals), which we may have also observed with the SEM micrographs (Figure 14O). Further research is needed to test the hypothesis that low VM charcoal can serve as a habitat for nitrifying bacteria and to determine differences in bacteria functionality or activity in soils amended with low VM charcoal. Secondly, the low VM charcoal itself contained less phenolic compounds than higher VM charcoals, which might inherently reduce the inhibition of nitrification (Chapter 2, Charcoal Characterization).

3.4.2 Implications

Our results provide a framework to interpret results of previous greenhouse studies, which showed that high VM content charcoals can have a negative effect on plant growth (Rondon et al., 2007; Deenik et al., 2010). In this study, we provide direct evidence that some high VM charcoals contain labile compounds which stimulate fungal growth and activity. Specifically, we studied the fraction of high VM corncob charcoal which was easily extracted with acetone. An analysis by GC-MS showed that this fraction consisted of phenolic and hydrocarbon compounds, which were not present in the low VM. When we removed this fraction from the charcoal, the initial effects of high VM charcoal on microbial activity during the first month of incubation were absent. Furthermore, we showed in the final incubation that the acetone-extractable compounds in high VM charcoal enhanced fungal growth and activity. Our findings provide compelling support that high VM charcoals can contain chemical compounds that are bioavailable and result in the stimulation of microbial activity. Therefore, higher losses of carbon from less carbonized charcoal are expected. This reduces carbon in charcoal for long term sequestration (Bird et al., 1999), while increasing microbial activity in the short-term (Steiner et al., 2007). However, centuries after charcoal additions, scientists have demonstrated enhancements in soil fertility (Glaser et al., 2002), richness in the microbial community (Kim et al., 2007), and the sorption of nutrients and organic carbons (Lehmann et al., 2005).

3.5 Conclusion

In conclusion, we showed that the high VM corncob charcoals enhanced microbial growth significantly more than low VM corncob charcoal. However, this was only true for one

feedstock. Our results show that VM content is a rough estimate of differences among charcoals and not sensitive enough to predict the behavior of charcoals derived from different feedstocks. Instead, the characterization of specific compounds using chemical fractionation and molecular analysis by GC-MS was required to describe charcoal behavior in soil. Though we provide primary support that high VM charcoals can contain a soluble fraction which enhances microbial activity and reduces nitrogen availability, a more complete characterization of the chemical composition of charcoals is still needed. Such knowledge will provide a better understanding of the differences in the chemical composition of charcoals derived from different feedstocks but with equivalent VM content. Ultimately, it will permit better predictions of the effects of various charcoals on plant growth and soil processes.

4. Effect of charcoal volatile matter content and feedstock on soil charge

4.1 Introduction

Highly weathered soils can be challenging for soil management. This is because such soils are typically characterized by variable charge due to their high levels of iron and aluminum oxides and amorphous materials. In variable charge systems, surface charge is determined by the ability of surface functional groups to accept or donate protons. The nutrient retention capacity is directly affected, since the cation exchange capacity (CEC) and anion exchange capacity (AEC) is dependent upon soil pH. Managers can improve CEC by liming soil to increase soil pH and generate negative charge or by adding organic matter, which carries high negative charge. Thus, it is critical for soil fertility managers of highly weathered soils to measure and manage CEC and AEC.

In the early investigations of the effect of charcoal on soil properties, findings showed that charcoal enhanced the CEC of highly weathered soils (Glaser et al., 2002). This has important implications since the retention of important plant nutrients is primarily dependent upon the soil's surface charge. Liang et al. (2006) reported that Brazilian Anthrosols, aged between 600 and 8700 year BP, contained high amounts of charcoal (black carbon or biochar) and almost a 2-fold greater potential CEC (at pH 7) than adjacent soils without black carbon. Charcoal particles isolated from these archaeological soils showed a greater charge density due to the oxidation of functional groups and surface adsorption of organic matter. More recent studies examining charcoal structure and the mechanisms responsible for the enhancement of its surface charge have shown that charcoal age and degree of weathering (oxidation) has a

significant effect on structure and surface charge (Cheng et al., 2006; Lehmann et al., 2005; Cheng et al., 2008; Nguyen et al., 2008). Cheng et al. (2008) showed that fresh charcoal carries positive charge. As charcoal ages in the natural environment (for more than 100 years), the surfaces showed an increase in elemental oxygen by the formation of carboxylic and phenolic functional groups, accompanied by a decrease in elemental carbon. Nguyen et al. (2008) also reported a rapid loss of carbon from charcoal within the first 30 years in soil, corresponding with a significant increase in carbonyl groups and oxygen bound to carbon (particularly on the charcoal surfaces). Lehmann et al. (2005) showed that the formation of carboxylic and phenolic carbon groups on the particle surface is structurally and spatially distinct from the inner core, the latter remaining highly aromatic even after thousands of years.

Like organic matter, charcoal exhibits variable charge properties (Cheng et al., 2008). However, unlike organic matter, which has a low zero point of net charge (ZPNC) and carries negative charge under typical soil pH, fresh charcoal has a relatively high ZPNC and typically generates a positive surface charge in pH ranges typical of tropical soils (Cheng et al., 2008). It is only with time that the charcoal particles develop a negative charge, or CEC, as the charcoal ages due to oxidation of the particle surfaces. The unique combination of the oxidized carbon functional groups and a high specific surface provides an opportunity for aged charcoal to significantly increase the CEC of highly weathered soils (Liang et al., 2006). However, previous studies have treated all charcoals as the same. Less attention has been devoted to examining the differential effect of feedstock and production conditions on the enhancement and development of CEC in soils. Prior studies have shown that fresh charcoal comprises of a wide array of materials, differing greatly in its chemical composition and its short-term effects on soil processes (Deenik et al., 2010).The objective of this study is to quantitatively determine the effect of fresh charcoal on soil charge. We aim to describe the charge of different charcoals and

their effect on the overall soil charge. Secondly, we will relate the results to previous studies of black carbon in the Brazilian Amazon. We hypothesize that fresh charcoal exhibits variable charge and will enhance the soil's CEC upon increasing oxidation. Charcoals with a higher VM content will undergo oxidation more rapidly than a relatively lower VM charcoal, thus high VM charcoals are expected to enhance CEC to a greater extent.

4.2 Materials and methods

4.2.1 Soil collection

We selected two soils for this study: the Halii Series (fine, ferruginous isothermic anionic acroperox) and the Leilehua Series (very fine, ferruginous, isothermic, ustic kanhaplohumult). The Halii Series was obtained from the University of Hawaii's Kauai Agricultural Research Center, Kapaa, Kauai Island (N22°06'53", W159.39'53"). The Halii Series is a highly weathered soil dominated by variable charge clay minerals with a pH of 4.88 under field conditions. These soils were selected based upon their highly weathered state and known AEC. The soil was air-dried, passed through a 2-mm aperture sieve, and stored under room temperature prior to laboratory experimentation. The Leilehua Series was an uncultivated, highly weathered Ultisol (Leilehua series, very fine, ferruginous, isothermic, ustic kanhaplohumult) collected from the 30-80 cm depth at the Wahiawa Correctional Facility, Mililani, Oahu Island (N21° 26'53".W157°57'52"). The Leilehua soil was selected because it is an extremely acid soil with low CEC, high exchangeable Al^{3+}, and a pH of 4.6. The soil was passed through a 6 mm aperture sieve in the field, placed in sealed 18.9 liter buckets to maintain field moisture, and transported to the greenhouse facilities of the University of Hawaii at Manoa. In the laboratory, soils were sieved once more to pass through a 2 mm sieve and stored in closed containers at 24°C in preparation for the laboratory experiments.

4.2.2 Charcoal collection

The charcoal feedstocks used in our laboratory studies included corncob husks, collected from Pioneer Seed Company on Oahu, and kiawe wood. The corncob charcoals were produced using the Flash Carbonization process developed at the Hawaii Natural Energy Institute of the University of Hawaii. This process involves the ignition and control of a flash fire at about 1 MPa within a packed bed of biomass. Heat released by the fire triggers the transformation of biomass into biocarbon with yields that can quickly reach the thermochemical equilibrium "limit" (Antal et al., 2003). In a typical run, peak temperatures after 40 minutes ranged from 300°C in bottom section of the reaction canister to just below 800°C in the upper section. We obtained two charcoal batches distinct in their thermal alteration, and specifically in their volatile matter content. The first type contained 23%. and the third, 7%. The kiawe charcoal was produced using traditional methods by a Maui-based charcoal company, and contained 23% volatile matter content.

4.2.3 Charge fingerprint

Our discussion of variable charge incorporates a variety of central concepts, outlined by Gillman (2007) and presented here.

- Variable charge can be negative or positive and includes the charge that develops primarily on surface hydroxyl groups by protonation and deprotonation processes. At low pH, protonation tends to occur, whereas deprotonation persists at high pH. As a result, positive charge develops at low pH, and negative at high pH.

- pH_0 is the point of zero net charge for all the variable charge components. This represents the pH at which there are equivalent amounts protonated and deprotonated sites, or positive and negative variable charge.

- Basic CEC comprises of the total amounts of basic cations adsorbed to the exchangeable sites at a specific solution pH and ionic strength.

- Total CEC includes basic and acidic cations adsorbed to the exchangeable sites at a specific solution pH and ionic strength.

- AEC is the total amount of anions that are adsorbed to the exchangeable sites at a specific solution pH and ionic strength.

- Zero point of net charge (ZPNC) is the point at which there is zero net charge in the entire soil system. At the ZPNC, total CEC equals AEC, as there are equal amounts of positive and negative charge. It also follows that pH_0 equals ZPNC in a totally variable charge systems (in absence of permanent charge by isomorphic substitution). In mixed systems, the existence of permanent negative charge of minerals with isomorphic substitution causes the ZPNC to be less than pH_0. In contrast, ZPNC exceeds pH_0 in the presence of permanent positive charge (Uehara and Gillman, 1980).

Gillman (2007) describes an analytical tool to characterize the charge dynamics in soils. Based on the principles by Uehara and Gillman (1980), this method distinguishes between CEC and AEC as soil pH and ionic strength is varied. It provides an easy and holistic approach to describe the various components of variable charge systems and predict the soil's cation and anion exchange capacities. We determined the charge fingerprint of the treated soils following a modified procedure of Gillman (2007).

1. One-gram of soil mixture was transferred into each of 6 to 9 preweighed 30-ml centrifuge tubes.

2. Ten-milliliters of 0.01 *M* $CaCl_2$ were added to each tube to saturate the exchange sites with calcium. Samples were then shaken on an end-to-end shaker for 2 hours.

3. After 2 hours, samples were centrifuged for 5 minutes at 8,000 rpm. The supernatant was carefully decanted with a pipet in order to minimize soil and charcoal loss. This step required precaution especially for charcoal samples since larger charcoal particles floated on the solution soil. Ten-milliliters of 0.002 *M* $CaCl_2$ were added to lower the ionic strength to 0.006. Samples were then vortexed. This step was repeated twice.

4. pH_0 was determined by adjusting the pH of the suspensions to a desired pH range (e.g. 2.5 to 6) with 0.1 *M* HCl or saturated $Ca(OH)_2$. After an overnight equilibration, the 0.002 *M* $CaCl_2$ suspension pH was recorded. Following this, 0.25 ml of 2 *M* $CaCl_2$ was added to each tube, and then shaken for 2 hours.

5. After 2 hours, the suspension pH (0.05 *M* $CaCl_2$) was recorded. The change (Δ) in pH was calculated by subtracting each pH obtained in step 4 (0.002 *M* $CaCl_2$) from each corresponding pH obtained in this step (0.05 *M* $CaCl_2$). The Δ pH was then plotted against the 0.002 *M* $CaCl_2$. pH_0 was determined from the point at which the change in pH is zero.

6. Step three was repeated twice to lower ionic strength.

7. The pH of the suspension was adjusted over a desired range (pH 3 to 6).

8. After an overnight equilibration, the suspension pH was recorded. Sampled were centrifuged again, decanted with pipet, and the supernatant was retained for calcium, aluminum, and chloride determination. The tubes were weighed to estimate the volume of entrained solution.

9. Following this, 20-ml of 1 *M* NH_4NO_3 solution was added. Samples were shaken for 2 hours to displace calcium, aluminum, and chloride from the soil surfaces. The samples were centrifuged again and the concentrations of calcium, aluminum, and chloride were determined. For each tube, the adsorbed amounts of calcium, aluminum, and chloride were calculated after subtracting entrained species. The total cation exchange capacity was estimated by the amounts of exchangeable calcium and aluminum at a given pH and ionic strength. However, the aluminum concentration was excluded for the Leilehua soils due to the presence of soluble aluminum. The anion exchange capacity is quantified by the amount of chloride adsorbed to the soil at a given pH and ionic strength. These CEC and AEC quantities were plotted against pH values recorded in Step 8. Total amounts of aluminum, calcium, and chloride in extracts were determined by the Agricultural Diagnostic Center Service at the University of Hawaii. Aluminum and calcium was measured with inductively coupled plasma spectrophotometer (ICP). Chloride was determined colorimetrically using an EasyChem Discrete Analyzer (DA).

Whereas the pH_0 represents the point of zero charge for the variable system, the ZPNC represents the pH in which there are equal amounts of positive and negative charge (AEC=total CEC). A complete soil charge fingerprint contains information for pH_0, CEC, AEC, and ZPNC. Only

the pH_0 was determined for incubated charcoal samples at selected sampling dates. We suspended the analysis at Step 8 in the protocol, since we experienced a continued loss of charcoal materials upon successive decanting steps. To minimize the effect of losing sample, we only report the pH_0. We determined pH_0 by fitting a quadratic regression and calculating the intersection of the x-axis (y=0). The CEC and AEC were fitted with exponential equations. The ZPNC was calculated from the point of intersection of CEC and AEC.

4.2.4 Incubation #1

In a charcoal aging experiment, we investigated the effects of charcoal solid particles (< 2 mm) and charcoal water extracts on charge properties of the Halii soil. We used three charcoal types, including 23% VM corncob, 7% VM corncob charcoal, and 23% VM kiawe charcoal. The charcoals were incubated for 10 weeks at approximately 60 °C without water additions. Destructive samples were obtained at 0, 5, and 10 week intervals. The solid charcoal materials were added to the soil at a rate of 2.5% (weight/weight on oven-dried equivalent), upon which the charge fingerprint was determined. The 2.5% rate was determined based upon typical agronomic rates for organic additions. Secondly, we tested the effect of the water soluble fraction of charcoal on soil charge, in order to determine if soluble compounds from the charcoal could sorb to soil particles and significantly alter soil charge. We obtained charcoal water-extracts by adding 30 ml of deionized water to 3 g of charcoal (oven-dried basis) following the procedure by Ghani et al. (2003). To obtain the water extracts, charcoal was shaken in deionized water for 30 minutes, centrifuged at 8000 rpm for 5 minutes, and then vacuumed filtered through a 0.45 micropore nitrocellulose filter paper. The charcoal extracts were added to the soil on a 5/1 (volume/soil weight) in order to saturate the soil particles with the extractant solution. The soil samples receiving the charcoal extract solution were then shaken

for 30 minutes, allowed to equilibrate overnight, and then shaken again for 30 minutes before centrifuged and supernatant removed. At this point, a charge fingerprint was determined for each of the treatments. Controls were also performed for soil alone and the soil after equilibrating with the addition of deionized water.

Charcoal samples were incubated for an additional three months in order to undergo wetting and drying cycles by adding water to reach 150% gravimetric water content on a biweekly basis. We had conducted the first three months without water to determine whether heat alone could sufficiently oxidize the charcoals and to prevent the solubility of charcoal materials in water. This incubation was extended for three months with additional heat and water treatment in order to replicate previous studies which included wetting/drying cycles (Cheng et al., 2006). We determined changes in the variable charge and pH_0. The charcoal samples were later added to the Leilehua soil to determine the effects of this more aggressive treatment on CEC, AEC, and ZPNC.

4.2.5 Incubation #2

In a second study, we performed an aging incubation of soil and charcoal mixtures. We continued our experimentation to determine whether it was more effective to incubate the charcoal in soil mixtures with heat and moisture treatments rather than heat only treatments as in the first incubation experiment. The Leilehua soil was mixed with the same suite of charcoals as in incubation #1 at a 2.5% (weight/weight) rate. Soil controls were also included. The mixtures were then incubated at approximately 60 °C for three month. During this incubation, deionized water was added biweekly to maintain 50% gravimetric water content. Therefore, the

mixtures underwent two wetting and drying cycles per week. Destructive samples were taken at 0, 1, 2, and 3 month intervals.

4.2.6 Charcoal application rate study

Finally, we performed a charcoal application rate study to determine the effects of increasing additions of non-incubated, fresh charcoal on the pH_0. We added 23% and 7% volatile matter corncob and 23% volatile matter kiawe charcoals to the Leilehua soil at 2.5, 5, 10, and 20% (weight/weight) additions. This extra step was taken to determine the rates and extent to which the soil's variable charge system could be altered upon the addition of charcoal.

4.2.7 Boehm titration

The Boehm titration (Boehm, 1994) was used to quantify various surface functional groups on charcoal particles. This procedure involves the reaction of basic solutions with different pK_b values with functional groups of differing pK_a values. A weak sodium bicarbonate solution was used to neutralize strong acid functional groups with pK_a of less than 6.37, which represents carboxylic groups. Secondly, a sodium hydroxide solution was used to neutralize acid functional groups with a pK_a of less than 15.74. The phenolic and lactonic groups were determined by the difference of acid functional groups neutralized by the two basic solution ($6.37<pK_a<15.37$). Briefly, 0.3 g of charcoal was added to closed containers with 15 ml 0.1 N standardized $NaHCO_3$ or NaOH basic solutions. The solutions were shaken for 24 hours, and then filtered through Whatman 42 filter paper. Aliquots of 5 ml of the filtered solutions were transferred to 10 ml of 0.1N standardized HCl. The resulting solutions were back-titrated with 0.1 N standardized NaOH, and calculations were performed to determine the quantity of surface acid functional groups ($mmol_c\ g^{-1}$charcoal) neutralized by the basic solutions. The Boehm

titration was performed on the charcoal particles, in triplicate, for all charcoals incubated at 60°C over the six month period (with and without wetting drying cycles). Samples were analyzed at 0, 5, and 10 weeks, and after an additional three months (after undergoing wetting and drying cycles).

4.2.8 Statistical analysis

The data for the Boehm titration were analyzed with non-nonparametric Kruskal-Wallis Multiple Comparison using Minitab 15. Separation of means was obtained using Dunn's Test.

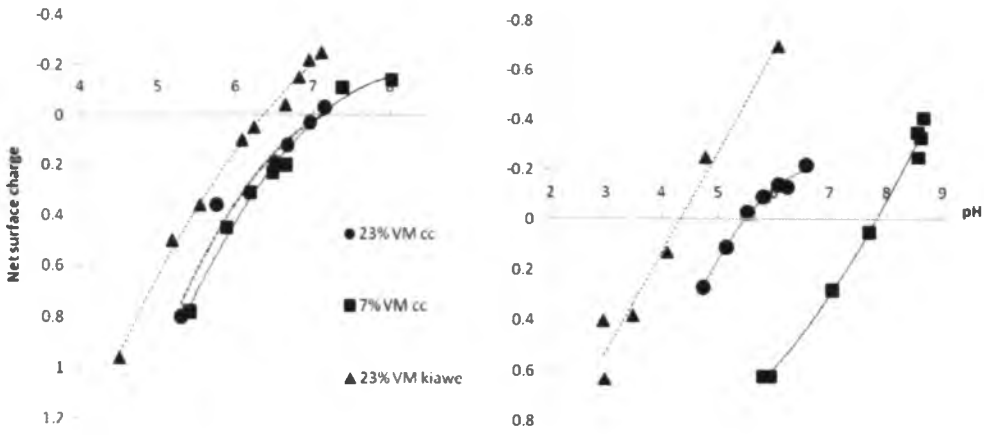
4.3 Results

4.3.1 Charge fingerprints

Charcoals

All charcoal materials exhibited variable charge (Figure 20). The pH_0, for variable charge systems, describes the point at which altering the ionic strength of the solution does not result in a corresponding change in solution pH. In other words, it is the point which there are equal number of protonated (positively charged) and deprotonated (negatively charged) sites, or the zero point of net charge of the variable charge components (Gillman, 2007). We found that the pH_0 for the fresh, non-incubated 23% VM corncob charcoal was approximately 6.98. In comparison, the pH_0 of the 7% VM corncob was 7.28, and 6.45 for the 23% VM kiawe. After six months under high heat conditions (60°C) and with biweekly wetting and drying cycles the values for pH_0 changed. In agreement with our hypothesis, pH_0 for the higher VM charcoals decreased to 5.60 for the 23% VM corncob char and to 4.30 for the kiawe char. In contrast, the pH_0 increased to 7.68 for the 7% VM corncob char. These results represent a 33% decrease in

pH_0 for the kiawe charcoal, a 20% decline for the 23% VM corncob, and a 5% increase for the7% volatile matter corncob charcoal.



A. Fresh Charcoal **B.** Aged charcoal for 6 months

Figure 20. Variable (pH dependent) charge on charcoal surfaces. The pH_0 shifted during six month incubation at 60°C with wetting and drying cycles (B) in comparison to the fresh charcoal. VM= volatile matter; cc= corncob charcoal; kiawe=kiawe charcoal. Lines were fitted using a quadratic regression, dotted lines for 23% VM cc; solid lines for 7% VM cc; and dashed lines for 23% VM kiawe charcoal.

Incubated charcoals and charcoal extracts added to Halii soil

The Halii soil control treatments exhibited a pH_0 of approximately 4.96, a ZPNC of approximately 5.38, a CEC ranging from 0.5 to 3.5 $cmol_c\ kg^{-1}$ and AEC ranging from 0.5 to 2.0 $cmol_c\ kg^{-1}$ within the experimental pH range (Table 8). The estimated CEC at pH 7 was 3.42 $cmol_c\ kg^{-1}$. It is interesting the note that the ZPNC of the soil is greater than its pH_0, which indicates the presence of minerals exhibiting permanent positive charge (Uehara and Gillman, 1980). Addition of the charcoals resulted in only slight changes in the soil charge fingerprint (Figure 21 and Table 8). The soil pH_0 increased upon the additions of all fresh charcoals and incubated charcoals for 5 and 10 weeks. There was no observable trend in pH_0 due to longer incubation or according to differences in charcoal VM content and feedstock. In general, the ZPNC of soil decreased with added fresh charcoal, but apparently increased upon incubation

with the exception of the kiawe at 10 weeks. The estimated CEC at pH 7 was increased slightly by the 23% and 7% VM corncob charcoals incubated for 10 weeks. It is important to note that the CEC measurements for the addition of fresh 23% VM corncob charcoal (0 weeks) to the Halii soil were almost of a magnitude greater than all other determinations. Experimental error is the only likely explanation, so we included these results from our analysis provided in Table 8.

In comparison to the charcoal additions, the charcoal water extracts resulted in an even greater increase in the soil pH_0, which was further enhanced upon the incubation of the charcoals (Figure 22 and Table 8). All charcoal water extracts reduced the ZPNC. In contrast to the charcoals, the estimated soil CEC at pH 7 declined upon the addition of water extracts from all charcoals incubated for 10 weeks.

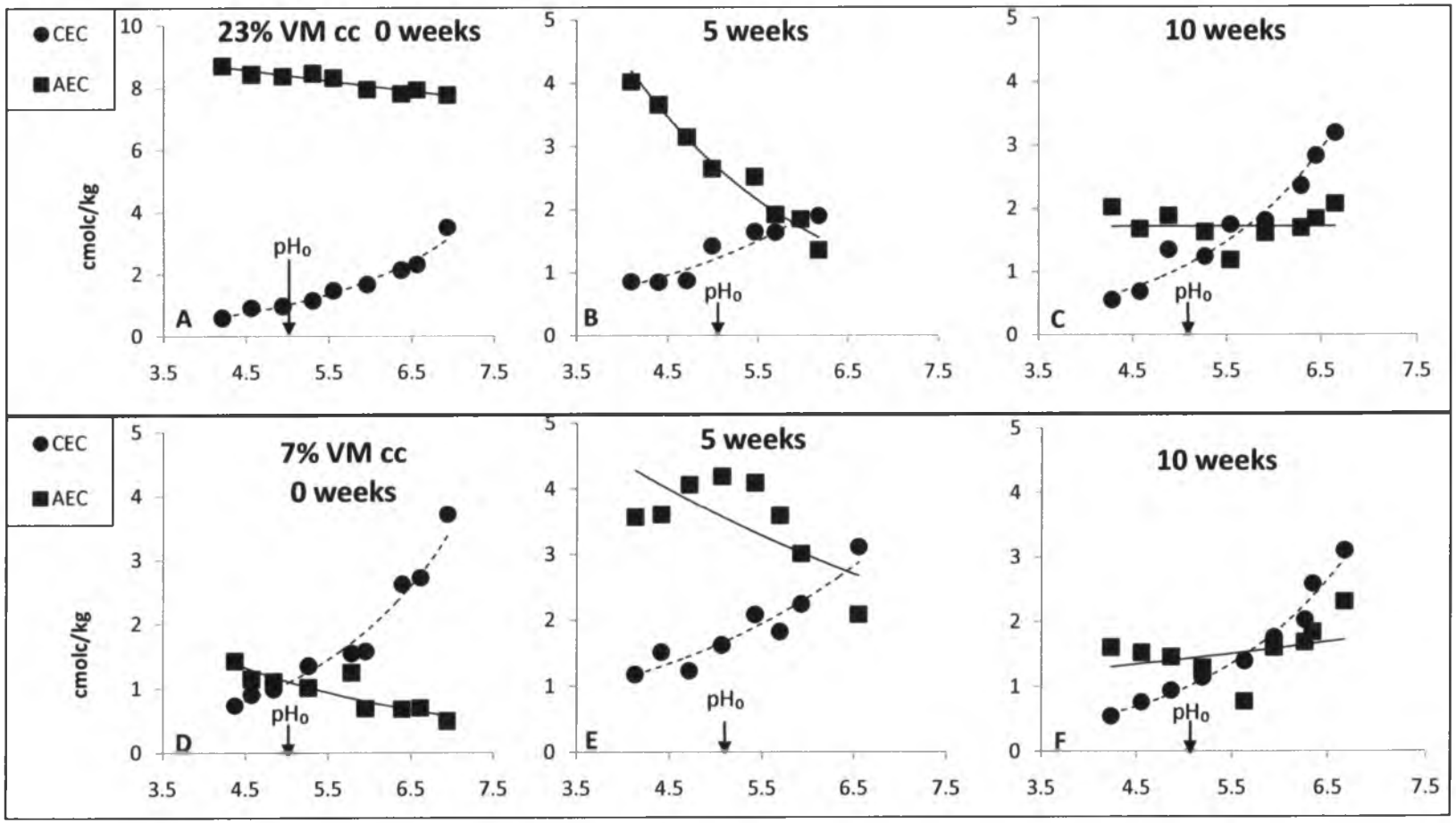


Figure 21 A-F. Charge fingerprints of Halii soil with added charcoal. Charcoals were incubated for 0, 5 or 10 weeks at 60°C prior to additions (2.5%). Charcoals included 23% VM (A-C) and 7% VM corncob (D-E). The circles represent CEC; squares, AEC; and triangles, variable charge (pH dependent) charge upon the particle surface. Lines were fitted using a exponential regression (dashed for CEC versus solid for AEC).

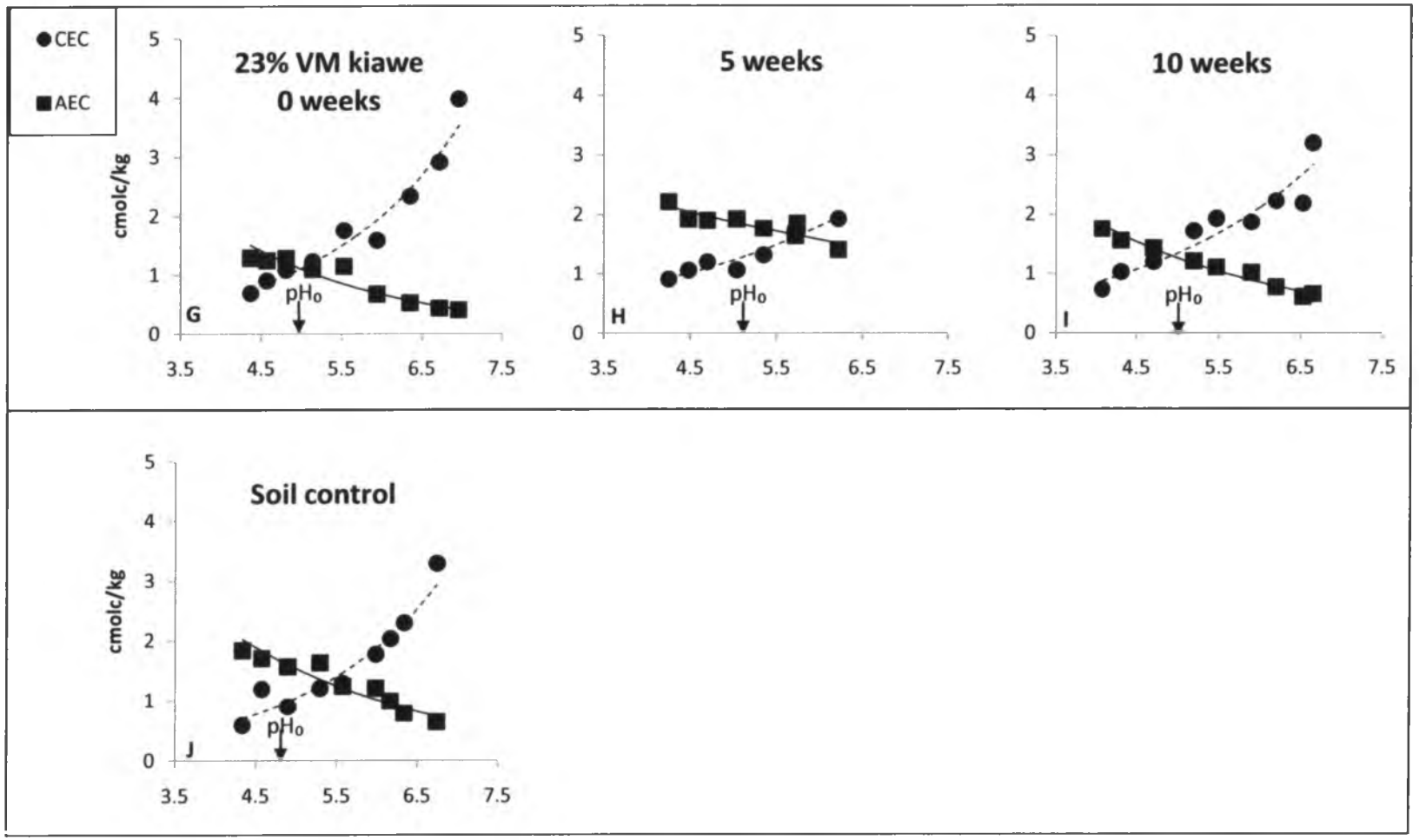


Figure 21 G-J. Charge fingerprints of Halii soil with charcoal additions. Charcoals were incubated for 0, 5 or 10 weeks at 60°C prior to additions (2.5%). The charcoals included 23% VM kiawe (G-I) and soil control (J). The circles represent CEC; squares, AEC; and triangles, variable charge (pH dependent) charge upon the particle surface. Lines were fitted using a exponential regression (dashed for CEC versus solid for AEC).

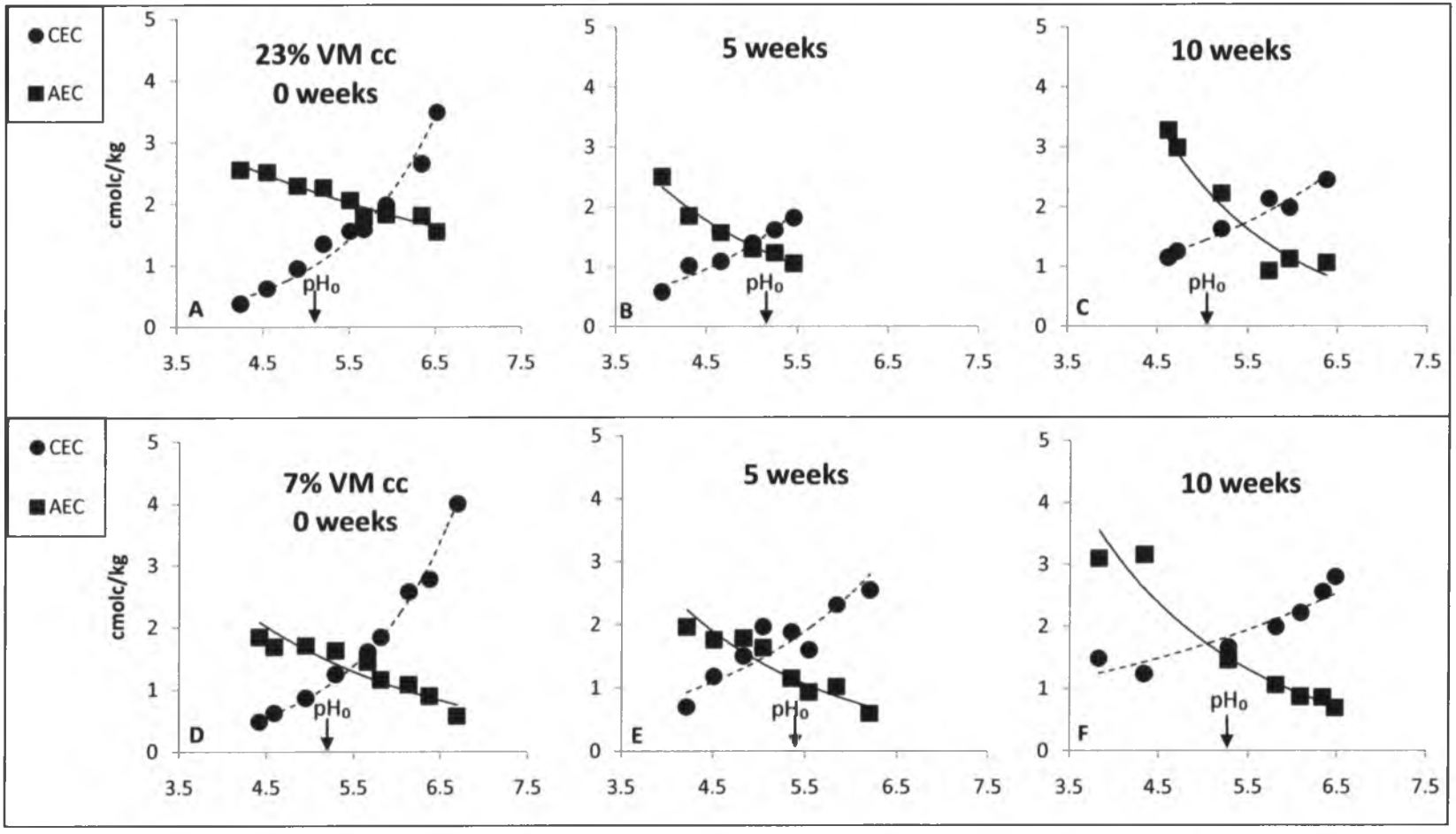


Figure 22 A-F. Charge fingerprints of Halii soil with additions of charcoal/water extracts. The charcoals included 23% VM corncob (A-C) and 7% VM corncob (D-F). The circles represent negative charge (CEC=cation exchange capacity); squares, positive charge (AEC=anion exchange capacity); and triangles, variable charge (pH dependent) charge upon the particle surface. Solid lines were fitted using an exponential regression (Dashed lines for AEC versus solid for CEC).

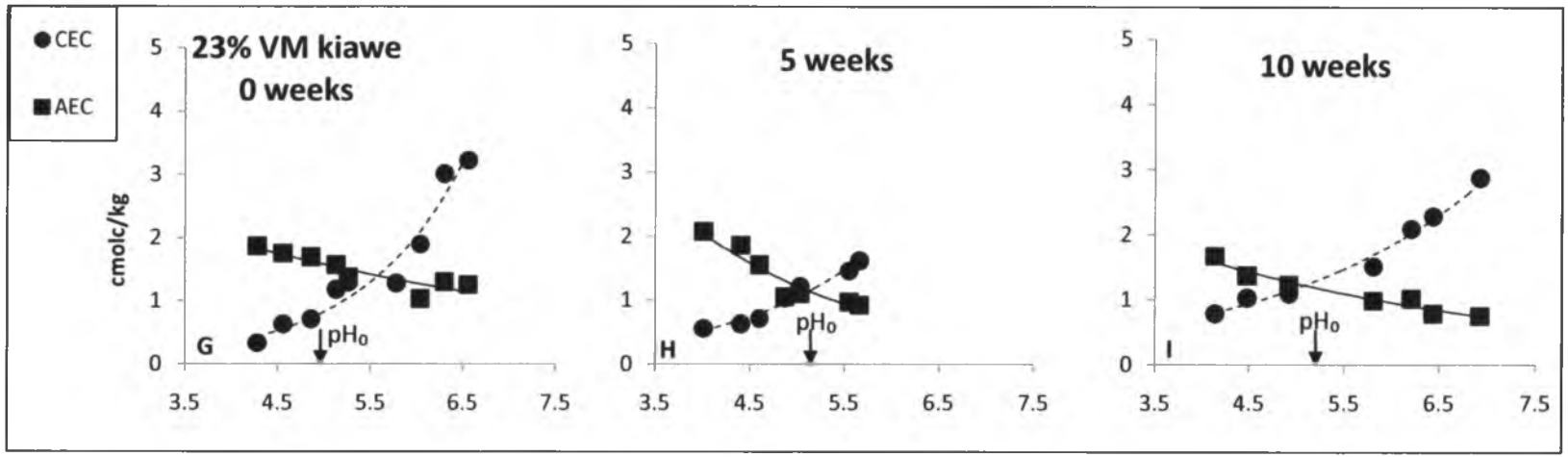


Figure 22 G-I. Charge fingerprints of Halii soil with additions of charcoal/water extracts. The charcoals included 23% VM kiawe charcoal (G-I). The circles represent negative charge (CEC=cation exchange capacity); squares, positive charge (AEC=anion exchange capacity); and triangles, variable charge (pH dependent) charge upon the particle surface. Solid lines were fitted using an exponential regression (Dashed lines for AEC versus solid for CEC).

Table 8. Values for pH_0, ZPNC, and estimated CEC pH 7 for incubated charcoals and their corresponding water extracts added to Halii soil

Charcoals								
	pH_0			ZPNC			CEC pH 7	
Charcoal	Fresh	Aged-5 wk	Aged-10 wk	Fresh	Aged-5 wk	Aged-10 wk	Fresh	Aged-10wk
7% VM corncob	4.97	5.22	5.15	5.03	6.42	5.69	3.49	3.71
23% VM corncob	5.05	5.19	5.06	NA	5.88	5.72	3.23	4.12
23% VM kiawe	5.01	5.14	5.10	4.99	5.76	4.93	3.61	3.32
Charcoal water extract								
7% VM corncob	5.20	5.37	5.30	5.45	4.98	5.04	5.32	2.9
23% VM corncob	5.09	5.17	5.09	5.82	4.99	5.44	5.35	3.26
23% VM kiawe	5.07	5.18	5.22	5.57	5.13	5.09	4.99	2.88
Halii soil	4.96			5.38			3.42	

Incubated charcoal and Leilehua soil mixtures

The Leilehua soil without charcoal additions had a pH_0 of approximately 4.23 and ZPNC of 3.26. Unlike the Halii soil, the ZPNC was generally less than its pH_0, indicating the presence of permanent negatively charged minerals. The CEC ranged from approximately 1.0 to 5.0 $cmol_c\ kg^{-1}$ for the experimental pH conditions, while the AEC ranged from 0.0 to 3.0 $cmol_c\ kg^{-1}$. The estimated CEC at pH 7 was 11.89 $cmol_c\ kg^{-1}$. The values for pH_0 decreased, while ZPNC increased slightly as the soils were incubated for three months; whereas, the estimated CEC at pH 7 decreased to 7.84 $cmol_c\ kg^{-1}$. The addition of the charcoals produced minor changes in the charge fingerprint parameters for the Leilehua soil during the three month incubation (Figure 23 and Table 9). In contrast to the charcoal effects observed in the Halii soil, the addition of each charcoal increased the soil pH_0 and ZPNC, with an increase in soil ZPNC by almost a full pH unit. However, aging of the charcoal caused the ZPNC to steadily decline throughout the incubation. The observed decline in ZPNC with time suggests an increase in net negative charge. These results were consistent when we added the charcoal which had been incubated for six months, but receiving water additions during the last three months (Figure 24).

The effect of fresh charcoal's net positive charge on soil charge was most observable in the rate study. With increasing additions of charcoals we detected an increase in the soil pH_0, which was most apparent with the low VM charcoal. Increasing the rate of charcoal showed only small effects on soil charge properties (Fig. 25). For the 23% volatile matter corncob charcoal applied at the 2.5% (weight/weight) rate the pH_0 was 4.16 and did not change at the 5% and 10% rate. However, at the 20% rate addition, the pH_0 increased slightly to 4.44. Likewise, the non-incubated 23% volatile matter kiawe charcoal did not result in a shift in the soil pH_0 at the 2.5%, 5%, and 10% rate additions, which remained around 4.3, but there was slight increase in

pH_0 to 4.48 at the highest application rate. The soil with the addition of the 7% volatile matter corncob charcoal had a pH_0 which ranged from 6.25 to 6.33 at the 2.5 and 5% rates; approximately 4.46 at 10% rate; and approximately 4.64 at the 20%.

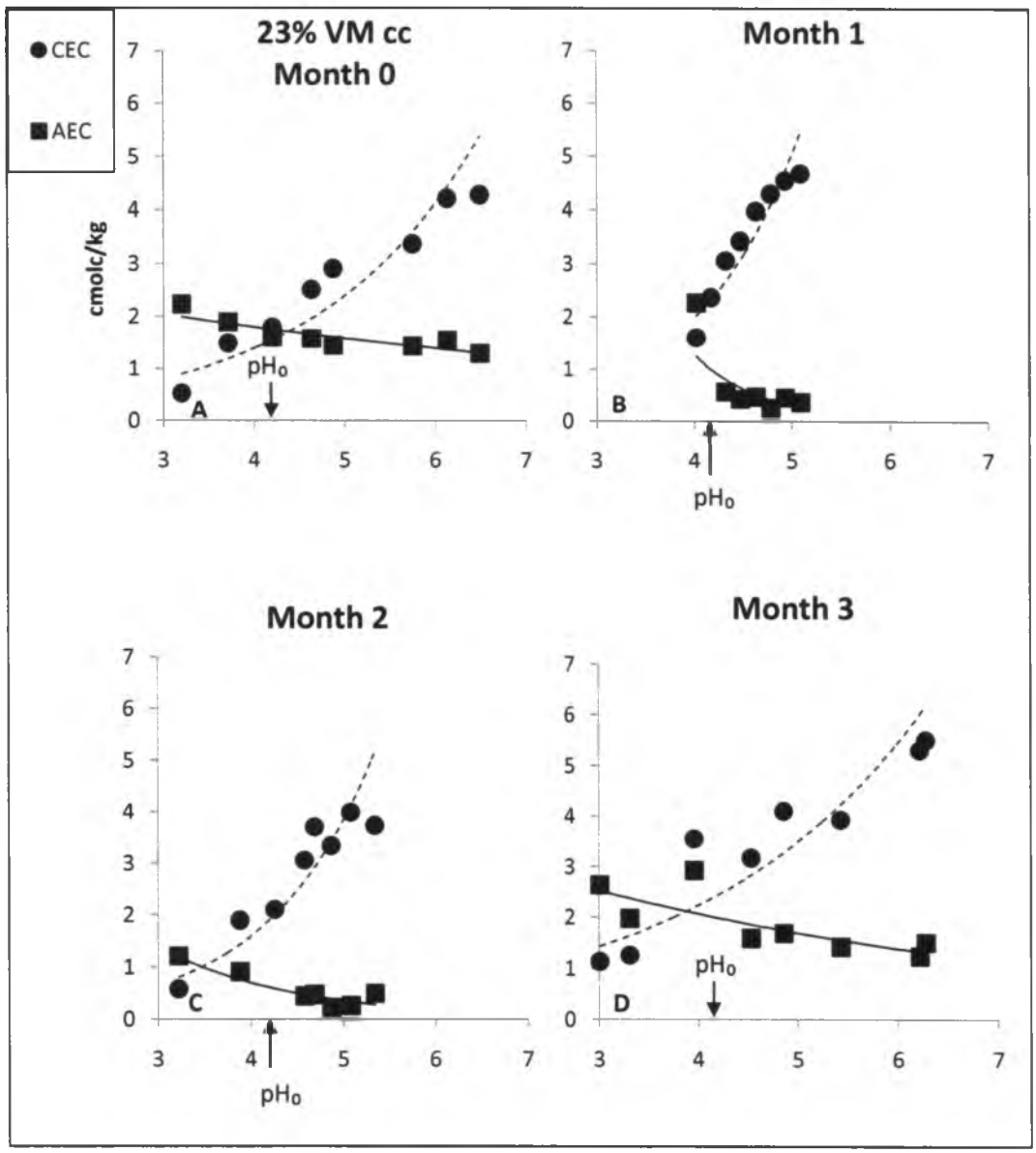


Figure 23 A-D. Charge fingerprints of Leilehua soil incubated with charcoal at 60°C and biweekly additions of 50% gravimetric water content. Charcoals were added to soil (2.5%, weight/weight). The charcoals included 23% VM corncob charcoal (A-D). The circles represent negative charge (CEC=cation exchange capacity); squares, positive charge (AEC=anion exchange capacity); and triangles, variable charge (pH dependent) charge upon the particle surface. Lines were fitted using an exponential regression (Dashed lines for AEC versus solid for CEC).

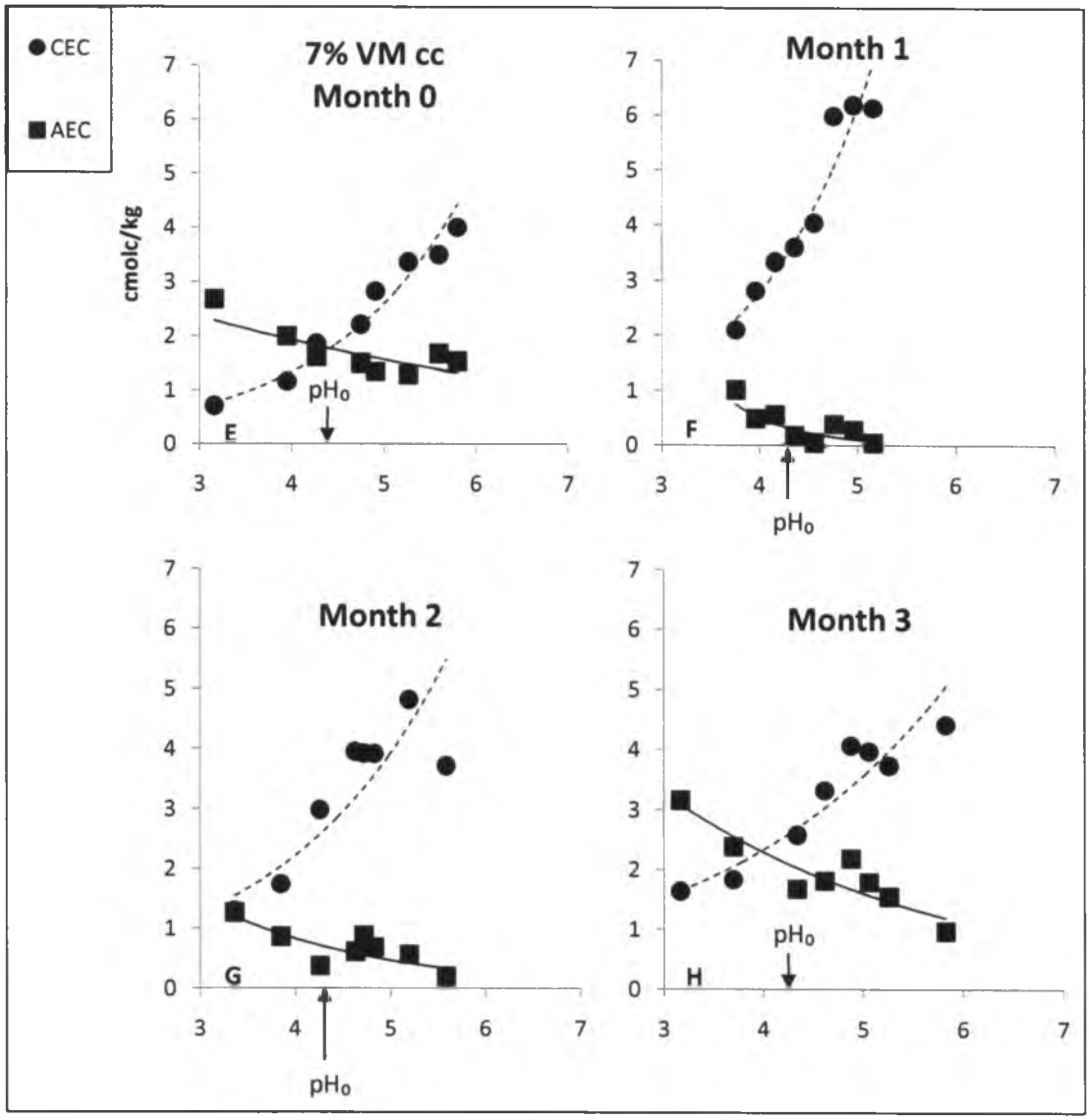


Figure 23 E-H. Charge fingerprints of Leilehua soil incubated with charcoal at 60°C and biweekly additions of 50% gravimetric water content. The charcoals included 7% VM corncob charcoal (E-H). The circles represent negative charge (CEC=cation exchange capacity); squares, positive charge (AEC=anion exchange capacity); and triangles, variable charge (pH dependent) charge upon the particle surface. Lines were fitted using an exponential regression (Dashed lines for AEC versus solid lines for CEC).

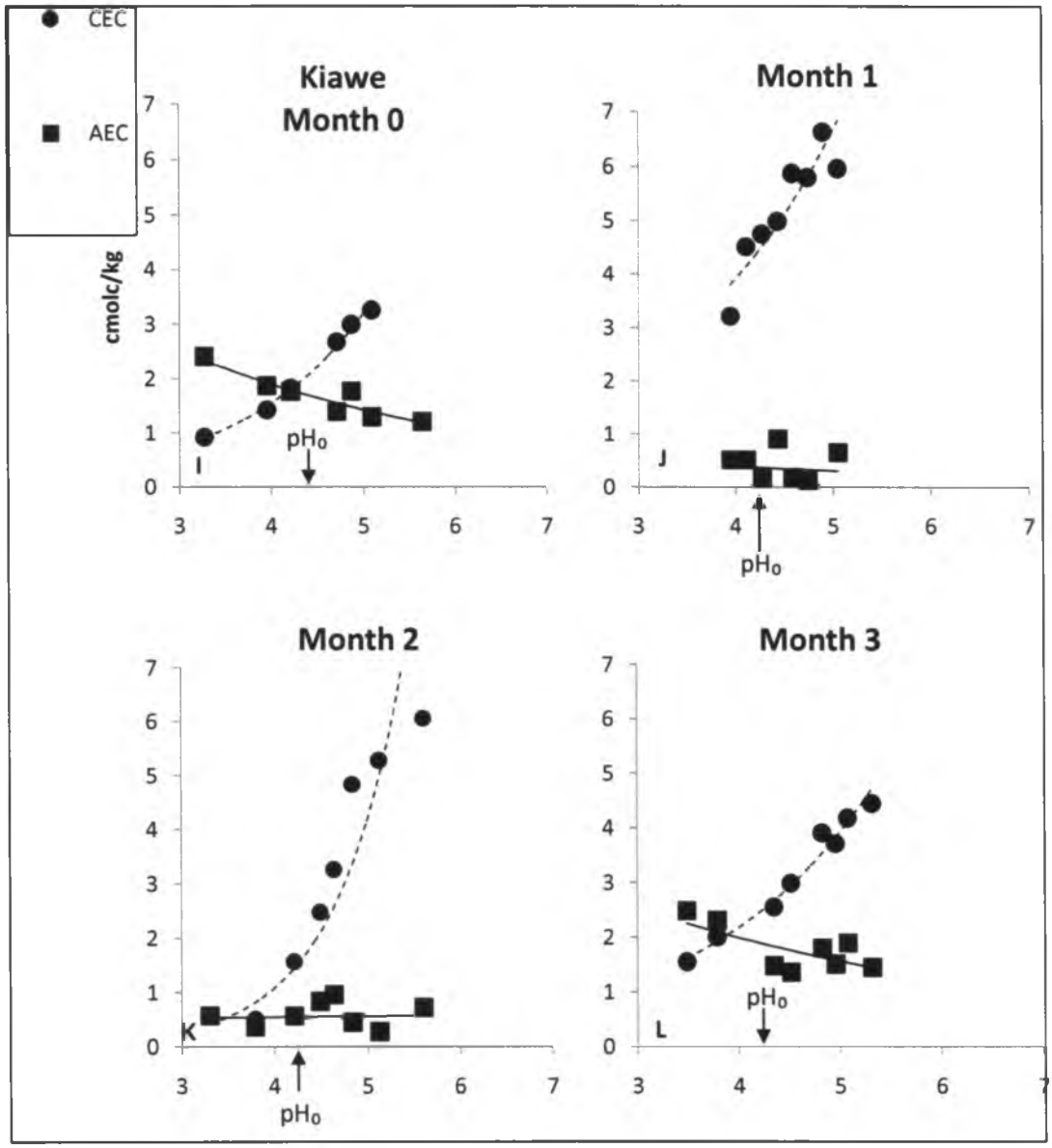


Figure 23 I-L. Charge fingerprints of Leilehua soil incubated with charcoal at 60°C and biweekly additions of 50% gravimetric water content. Charcoals were added to soil (2.5%, weight/weight). The charcoals included 23% VM kiawe charcoal (I-L). The circles represent negative charge (CEC=cation exchange capacity); squares, positive charge (AEC=anion exchange capacity); and triangles, variable charge (pH dependent) charge upon the particle surface. Lines were fitted using an exponential regression (Dashed lines for AEC versus solid for CEC).

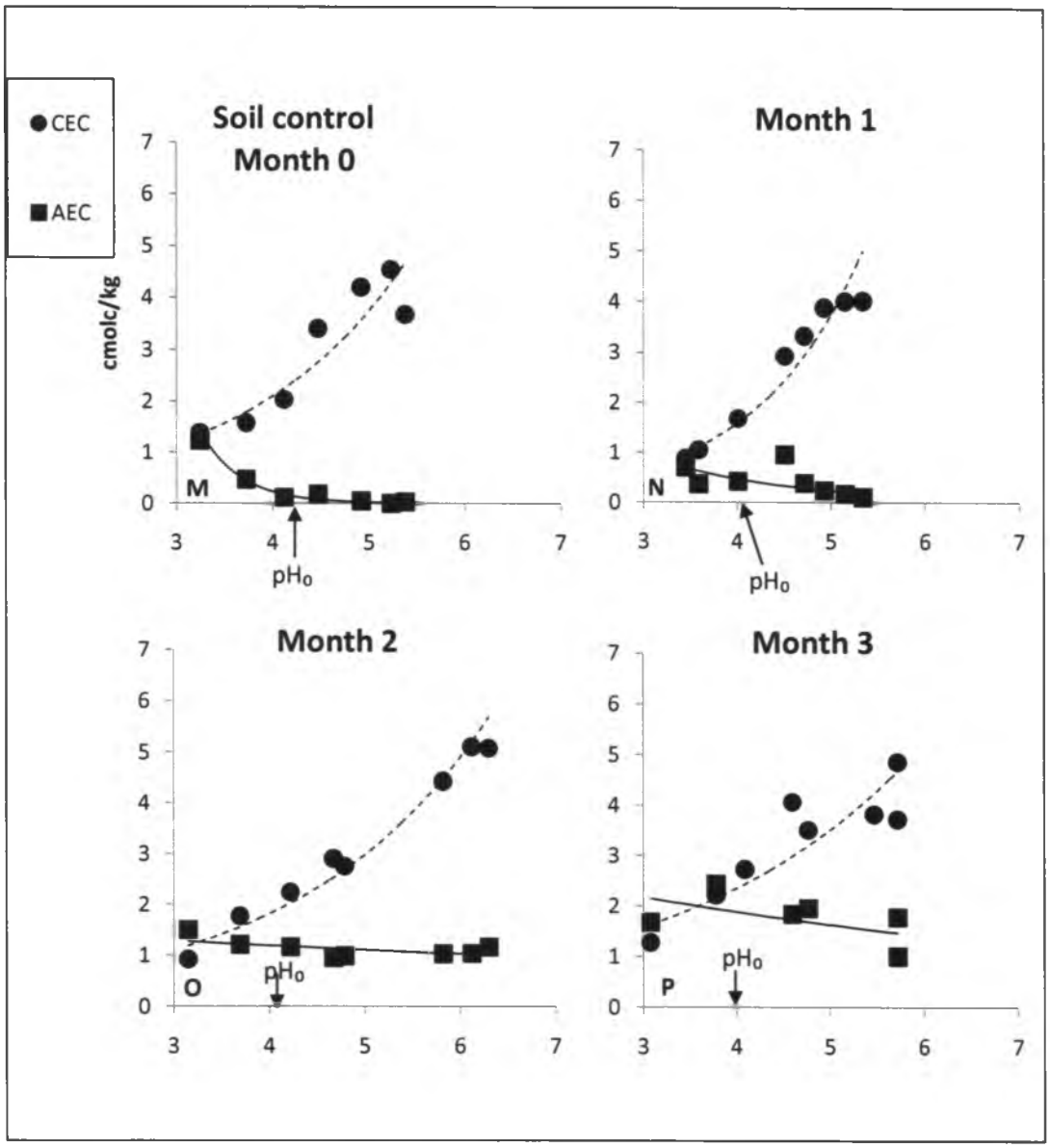


Figure 23 M-P. Charge fingerprints of Leilehua soil incubated without charcoal at 60°C and biweekly additions of 50% gravimetric water content. Figures M-P represent soil control without added charcoal. The circles represent negative charge (CEC=cation exchange capacity); squares, positive charge (AEC=anion exchange capacity); and triangles, variable charge (pH dependent) charge upon the particle surface. Lines were fitted using an exponential (Dashed lines for AEC versus solid lines for CEC).

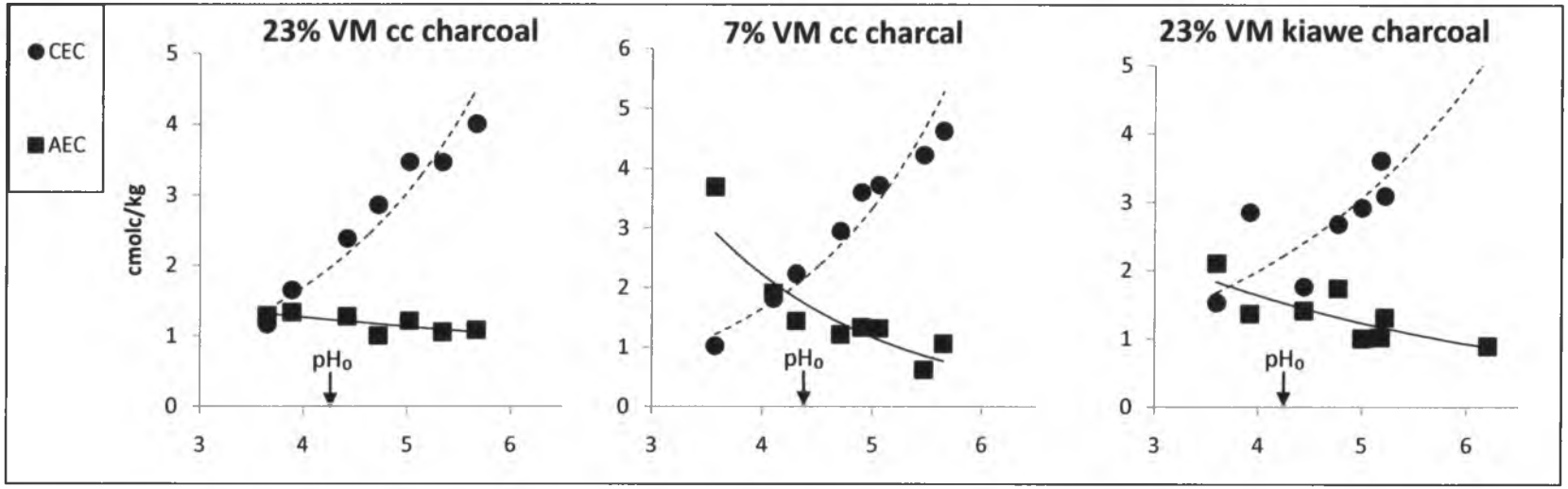


Figure 24. Charge fingerprints of Leilehua soil receiving additions of charcoals that were incubated for six months at 60°C with biweekly additions of 150% gravimetric water content during the last three months. Charcoals were added to soil included 23% and 7% VM corncob, 23% VM kiawe charcoal. The circles represent negative charge (CEC=cation exchange capacity); squares, positive charge (AEC=anion exchange capacity); and triangles, variable charge (pH dependent) charge upon the particle surface. Lines fitted using an exponential regression (Dashed AEC, Solid CEC).

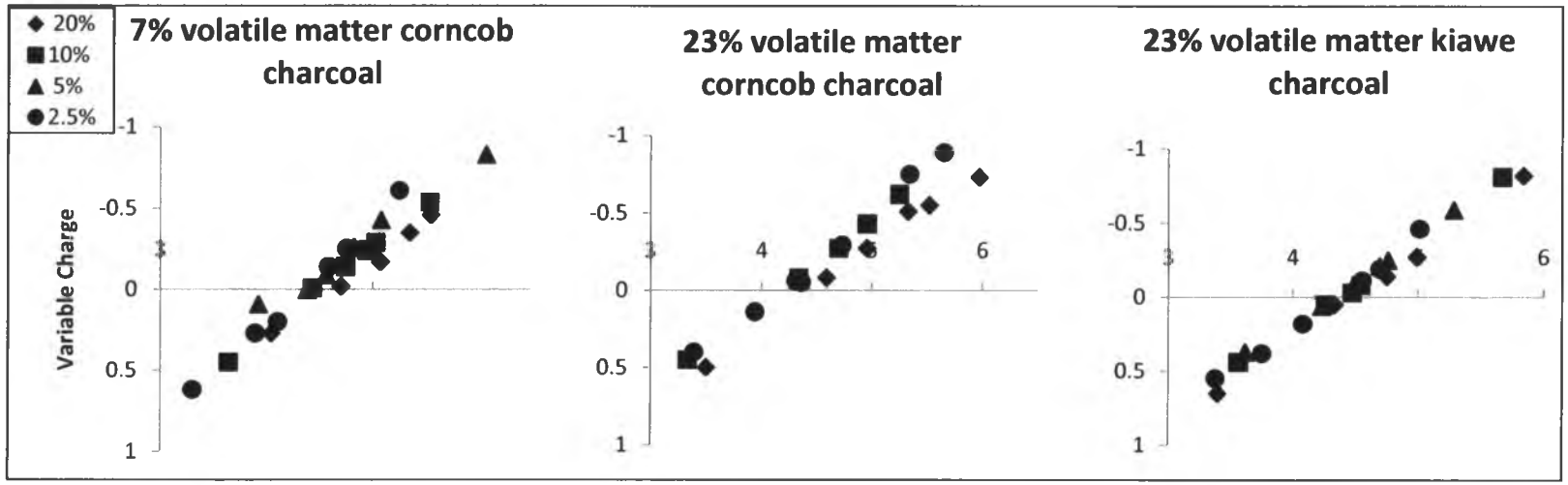


Figure 25. Application rate study showing increasing rates (2.5% represented by circles; 5%, triangles; 10%, squares; 20%, diamonds) of charcoal additions to Leilehua soil.

Table 9. Values for pH_o, ZPNC, and estimated CEC pH 7 for incubated Leilehua mixed with charcoals

	pH_o				ZPNC				CEC pH 7 ($cmol_c\ kg^{-1}$)	
Charcoal	Fresh	Aged-1 mo	Aged-2 mo	Aged-3 mo	Fresh	Aged-1 mo	Aged-2 mo	Aged-3 mo	Fresh	Aged-3 mo
7% VM corncob	4.38	4.27	4.29	4.23	4.42	3.51	3.14	3.98	9.83	8.31
23% VM corncob	4.18	4.15	4.18	4.15	4.38	3.82	3.47	3.88	7.09	8.54
23% VM kiawe	4.38	4.25	4.26	4.27	4.20	3.57	3.48	3.90	13.55	12.65
Soil alone	4.23	4.04	4.05	3.96	3.26	3.22	3.27	3.59	11.89	7.84

4.3.2 Boehm titration

Results for the Boehme titration (Table 10) showed that the fresh 7% volatile matter corncob charcoal had the highest total surface charge with total acid functional groups exhibiting 4.87 $mmol_c\ g^{-1}$; carboxylic functional groups, 3.48 $mmol_c\ g^{-1}$; and phenolic and lactonic groups, 1.38 $mmol_c\ g^{-1}$. In comparison, the 23% volatile matter corncob had 4.25 $mmol_c\ g^{-1}$ of total acidic functional groups, 3.57 $mmol_c\ g^{-1}$ of carboxylic, and 0.68 $mmol_c\ g^{-1}$ of phenolic and lactonic groups. The 23% volatile matter kiawe had no measurable charge associated with phenolic and lactonic groups, and 3.1 $mmol_c\ g^{-1}$ carboxylic groups. After 10 weeks of incubation at 60°C, the 7% volatile matter corncob showed no increase in total functional groups, but showed a relative decline in carboxylic (2.90 $mmol_c\ g^{-1}$) and increase in charge associated with phenolic and lactonic groups (1.90 $mmol_c\ g^{-1}$). The 23% volatile matter corncob, too, showed a decline of carboxylic groups (2.73 $mmol_c\ g^{-1}$) and increase in phenolic groups (1.17 $mmol_c\ g^{-1}$). The 23% volatile matter kiawe followed this trend as well, with carboxylic groups declining to 2.5 $mmol_c\ g^{-1}$, and phenolic and lactonic increasing to 0.3 $mmol_c\ g^{-1}$.

All charcoals were incubated for an additional three months, with biweekly additions of water to attain 150% gravimetric water content. The 7% volatile matter corncob showed the same amount of carboxylic groups of 3.0 $mmol_c\ g^{-1}$ as after 10 weeks of high heat, alone, but phenolic and lactonic groups decreased in charge by about 0.75 $mmol_c\ g^{-1}$. For both the 23% volatile matter corncob and kiawe charcoals, the same amount of carboxylic groups was measured relative to the first 10 weeks after the additional three month incubation. However, in both cases, no phenolic and lactonic groups could be measured.

Table 10. Quantification of surface functional groups of charcoals by the Boehm titration after incubated for 0, 5, or 10 weeks at 60°C, with an additional 3 month incubation with water additions. Charcoals included 23% VM (volatile matter) cc (corncob charcoal), 7% VM cc, 23% VM kiawe charcoal, and activated charcoal.

		Carboxylic	Phenolic and lactonic	Total
			mmolc/g	
23% VM cc	0 weeks	3.57a ± 0.09	0.68ab ± 0.08	4.25abc ± 0.03
	5 weeks	3.30ab ± 0.17	0.97ab ± 0.18	4.27abc ± 0.03
	10 weeks	2.73ab ± 0.29	1.17ab ± 0.32	3.90abc ± 0.06
	6 months	2.77ab ± 0.03	<0.00ab ± 0.03	2.60bc ± 0.00
7% VM cc	0 weeks	3.48ab ± 0.19	1.38ab ± 0.13	4.86ab ± 0.07
	5 weeks	3.07ab ± 0.12	1.83a ± 0.12	4.90a ± 0.00
	10 weeks	2.90ab ± 0.06	1.90a ± 0.15	4.80a ± 0.20
	6 months	3.03ab ± 0.20	1.17bab ± 0.13	4.20abc ± 0.06
23% VM kiawe	0 weeks	3.13ab ± 0.03	<0.00b ± 0.07	2.90abc + 0.06
	5 weeks	2.83ab ± 0.09	<0.00ab ± 0.09	2.80abc ± 0.12
	10 weeks	2.90ab ± 0.26	0.30ab ± 0.53	2.80abc ± 0.27
	6 months	2.63b ± 0.03	<0.00b ± 0.07	1.50c ± 0.09
Activated charcoal		3.40ab ± 0.06	0.97ab ± 0.97	4.37abc ± 0.17

Values represent the mean (n=3) ± SE. Values in the same column with the same letters are not significant different based upon Kruskal-Wallis Multiple Comparisons with Dunn's Test.

4.4 Discussion

We confirmed our first hypothesis that aging has a differential effect on the charge properties of charcoals with varying VM content. Specifically, we found that high VM charcoals showed a decrease in pH_0, and thus an increase in net negative charge upon aging. Our results show that the variable charge of charcoals can be altered during a short-term incubation with heat and moisture. Particularly, we found that the pH_0 of the 23% VM charcoals decreased with increasing oxidation. We observed a decline in 23% VM kiawe charcoal pH (in 0.002 *M* $CaCl_2$) by approximately 1.4 units and by more than 0.5 unit in 23% VM corncob charcoal (data not shown). This corresponded with a decrease in pH_0 by 20 to 33%. Our results are in agreement with Cheng et al. (2006), who saw a decline in charcoal pH (in water) from 5.4 to 4.1 during a four month incubation at 70°C and 50% water holding capacity. A subsequent short-term aging experiment performed by Cheng et al. (2008) showed that the ZPNC of the fresh charcoals decreased by more than 50% during incubation. Cheng et al. (2006) also reported increase in potential CEC from 140 to 894 $mmol_c\ kg^{-1}$ charcoal.

While we were able to confirm the variable charge nature of charcoals and measure an aging effect on high VM charcoals, we were unable to show any substantial effect on soil ZPNC and CEC when these charcoals were applied to two soils with inherently low CEC. This is in remarkable contrast to the findings of Cheng et al. (2006), who showed that the potential CEC of the soil was improved by more than four-fold when charcoal was mixed with soil (4% weight/weight) and incubated at 70°C . We expected to support this study by showing a large increase in soil CEC since the charcoals had developed more net negative charge during our incubation. However, despite the observed changes in variable charge characteristics, no

additions of charcoal at the 2.5% rate (weight/weight) affected the charge fingerprint of the Leilehua or Halii soil regardless of incubation time, volatile matter content, or feedstock. Differences in methodology may explain the discrepancy in results. Cheng et al. (2006) measured the CEC at pH 7, which encompasses potential CEC. Since both charcoals and highly weathered tropical soils are characterized by variable charge systems, this method grossly overestimates the CEC and is not representative of CEC at the soil's field pH. We believe that this finding should not be overlooked, since it is not agronomically viable to raise the pH of a Leilehua or Halii soil to pH 7. Liming curves show that the Leilehua soil requires approximately 6.5 tons of $CaCO_3$ $acre^{-1}$ to raise the soil pH to 7, and more than 8 tons of $CaCO_3$ $acre^{-1}$ for the Halii soil (Hue and Ikawa, 1997). We recommend that investigations of the effect of charcoal on soil CEC should be carried out at the natural pH of the soil. Secondly, whereas Cheng at al. (2006) subjected the charcoal materials to intensive leaching to remove adsorbed and readily soluble materials, we did not leach the charcoal prior to the incubations and the determination of charge fingerprint. We did not leach the charcoal since the VM content can include recondensed volatiles on the surface of charcoal surface (Meszaros et al., 2007). The process of leaching could remove adsorbed compounds and thus change the surface characteristics of charcoals. The effect caused by leaching high VM charcoals should be investigated.

Our study is the first to show that VM content influences the oxidation process of charcoal and its charge characteristics. Both high VM charcoals in our experiment showed a decrease in pH_0 by at least 20%. Since the fresh charcoal used in the Cheng et al. (2006) study largely resembles the NMR and FTIR spectra of our 23% volatile matter charcoals (Chapter 2, Charcoal Characteristics), it is likely that their results are representative of a higher VM charcoal rather than a lower VM charcoal. These results confirm the hypothesis, presented in Cheng et al. (2008), that the oxidation rates for less thermally altered charcoals are more rapid. Our

results showed that the pH_0 of the low (7%) VM corncob charcoal increased by approximately 5% and the pH (in water) increased by 1.0 unit during the course of the incubation. Though we are unable to identify the mechanism, it is apparent that the charcoals are behaving differently. This might be due to the presence of dissimilar materials in the charcoals, for instance ash in the low VM charcoal and tarry materials in the high VM charcoals.

Results for the Boehm titration were not very clear. We expected to show an increase in carboxylic and phenolic groups on the surface of charcoals with time, particularly in the high VM charcoals. Prior to incubation, we showed that the lower VM charcoal had greater total surface acid functional groups, which might be due to its greater surface area (Antal and Gronli, 2003). In agreement, our NMR data from the characterization work showed that the low VM charcoal contained greater carboxylic acid groups. We observed similar quantities of phenolic and lactonic groups for the fresh charcoals as Cheng et al. (2006), but measured ten-fold more carboxylic groups. Despite this difference, we did not show increases in CEC. During the incubation at 60°C, we showed that the phenolic and lactonic groups almost doubled for the 23% VM corncob, in agreement with Cheng et al. (2006). The 7% VM corncob charcoal also underwent an increase in phenolic and lactonic surface groups, but to a lesser extent, whereas the kiawe charcoal showed the appearance of phenol and lactonic groups within this period. However, in direct contrast to Cheng et al. (2006), we showed a decline in carboxylic groups for all charcoals (23% and 7% VM corncob and 23% VM kiawe) with time. Furthermore, when water was added to the incubations for an additional three-month period, all charcoals even exhibited a decline in total acidic functional groups. Though we supported our hypothesis that high VM would oxidized more quickly, by showing a greater development of phenolic groups during the incubation, we unexpectedly showed a decline in carboxylic groups for all charcoals. One explanation for these divergent results is that we did not leach the charcoals with water prior to

the experiment in order to remove the ash and tar products like Cheng et al. (2006). The Boehm titration works best with hydrophilic charcoals. The 23% volatile matter charcoals used in our experiment have hydrophobic properties, while the 7% volatile matter charcoal is relatively hydrophilic but contains higher ash content. Knicker (2007) describes the formation of a hydrophobic layer in soil after a forest fire due to the greater stability of lipid and lignin derivatives. The Boehm titration may not be a suitable procedure to estimate surface functionality for charcoals with bio-oils, minerals and ash present (Lehmann and Joseph, 2009). Therefore, this method is not appropriate for charcoals with high VM and ash content due to interference during the measurement. An additional study must be performed to determine (1) whether the removal of these materials with leaching would eliminate such interference and (2) thus be responsible for the discrepancies in our results.

Our interest in determining the effect of charcoal on soil charge properties is derived from work performed in the Brazilian Amazon, where reports show that archaeological soils amended with charcoal exhibit higher CEC (pH 7) than adjacent soils (Liang et al., 2006; Glaser et al., 2002). Previous research attributes the high CEC to the development of carboxylic and phenolic groups along the aromatic charcoal backbone with increasing oxidation with time. However, it is important to acknowledge that the management of these archaeological soils involved a mixture of amendments, including green wastes, fish bones, and charcoal materials. In an attempt to replicate this effect in a Ferralsol with charcoal from secondary forests, Lehmann et al. (2002) showed that effective CEC of the soil more than quadrupled with the addition of charcoal. However, these researchers estimated ECEC by summation of exchangeable cations and acidity, which is an indirect measurement of exchange sites. The summation of cations is an unreliable estimate since it is dependent upon extraction efficiency and overestimates CEC by including soil solution cations in its measurement. Furthermore, the

effective CEC was only enhanced by 25% in comparison to the soil receiving fertilizer only. Other studies reporting charcoal's ability to increase effective CEC were conducted in sandy or poorly developed soil (Glaser et al., 2002), and thus are not comparable to our study which included clay soils with high potential surface charge. Charcoals are known to have great surface area and charge density (Liang et al., 2006), which could greatly contribute to the charge of a sandy soil with low specific surface. In comparison, highly weathered soils of Hawaii are rich in variable charge clay minerals such as kaolinite, aluminum and iron oxides, and amorphous materials. Unlike sandy soils, these soil components can have very high surface area and potential charge, but typically exhibit low CEC under naturally acidic conditions due to their pH dependent charge. For instance, the Leilehua soil used in this study had a potential CEC of more than 10 $cmol_c\ kg^{-1}$, which is even greater than the CEC pH 7.0 of only 2.5 $cmol_c\ kg^{-1}$ in Cheng et al. (2006). It is conceivable that the charcoal's pH_0 would have to decrease to pH 2 or 3 (similar to soil organic matter) in order to significantly and positively contribute to soil CEC.

Results from our laboratory study involving fresh charcoals questions the agronomic value of charcoal as an amendment in soils with a high surface charge potential. First, fresh charcoal has very different variable charge properties than aged charcoal. Specifically, we showed that fresh charcoal has a pH_0 between 6.5 and 7.0; and when it is added to soil at a very high rate (20% weight/weight), it actually increased the soil's pH_0. Thus, fresh charcoal increases the positively charged exchange sites of the soil, rather than the negative. Secondly, fresh charcoal is expected to exhibit net positive charge at the field pH of an acidic, highly weathered soil. Thirdly, our study shows that the addition of 2.5% (weight/weight) charcoal had only small effects on the ZPNC, CEC, and AEC of the soil, regardless of charcoal volatile matter content and feedstock. In short, we have found no evidence that charcoals produced by a modern pyrolitic method can improve the fertility of highly weathered Hawaii soils with low CEC

in the short-term. Further research is needed, specifically to determine the rates, the age, the characterization of the charcoal, as well as the preparation of the charcoal (e.g. prior leaching) which would positively enhance soil CEC at the field pH of soil.

4.5 Conclusion

In conclusion, the suitability of charcoal for enhancing the CEC of Hawaii soils is questionable. While we showed that high VM charcoals developed net negative charge in short-term incubation, this did not translate into substantial increases in the CEC of two Hawaii soils. Our study, instead, raises additional questions. First, further investigation is needed to identify the mechanism responsible for the differential charge development of low VM and high VM charge. Secondly, we must verify that the presence of ash and tar materials in the charcoal is responsible for the interference caused during the determination of surface acid functional groups. This can be accomplished by leaching the charcoal prior to surface charge quantification. Finally, a detailed comparison of the potential charge systems of charcoals and Hawaii soils must be performed to show whether their similarities are preventing substantial increases in CEC.

5. Conclusions

The primary goal of our research was to characterize the VM content of charcoals and relate differences in charcoal VM content to soil processes. We were mainly interested in VM content, because it is an easily-measured property of charcoal with an inverse relationship to carbonization temperature. Previous greenhouse studies have shown that VM content had a significant effect on plant growth and nitrogen uptake. Based upon findings of our previous work, we hypothesized that high VM charcoal contained a labile carbon pool which enhanced microbial activity and the immobilization of nitrogen. In a series of laboratory studies, high VM charcoals that contained extractable labile carbon simulated microbial activity and nitrogen immobilization in soil. In a second series of experiment, we showed that high VM charcoals developed net negative charge more quickly upon aging than a low VM charcoal. These findings have improved our capacity to predict how charcoals with varying VM content influence important aspects of soil fertility.

In our first study, we showed that the chemical structure of charcoals of high VM charcoal differs greatly from low VM charcoals. NMR analysis suggested that the VM content of charcoals, regardless of feedstock, consists primarily of alkyl carbon and oxygen-substituted alkyl carbons. A measurable fraction of the non-aromatic carbon groups in the high VM charcoal were also soluble in water. The FTIR spectra showed that the functional groups in the VM fraction included CH aliphatic carbons, ether linkages, and C-O bonds. In comparison, the low VM charcoal contained much less functional groups, more aromatic carbon, slightly more carboxylic groups, and less water soluble components. More detailed analysis of chemical composition using GC-MS showed that the acetone-soluble fraction of high VM corncob

charcoals (63 and 23%) contained phenols and fatty acid derivatives, which decline upon increasing thermal alteration, or charring severity.

One of our most interesting findings is that we did not detect any compounds in the 23% VM kiawe charcoal with GC-MS, despite containing greater concentrations of total phenolics as measured by Prussian Blue. Presumably, the "volatile matter fraction" is not adequately characterized by our fractionation of extractable charcoal compounds with acetone and subsequent analysis with GC-MS. We propose the utilization of other solvents to extract different carbon pools in charcoal and additional instrumentation, such as Py-GCMS and HPLC, for a more detailed description of the chemical composition of such charcoals.

In our second set of experiments, three incubation studies showed that the bioavailability of charcoal is contingent upon its VM content or degree of thermal alteration. We found that for a given feedstock, VM content was related to the relative propensity of the corncob charcoals to stimulate microbial activity and influence N dynamics. However, there are limitations in interpreting VM content for charcoals derived from different feedstocks. For instance, the 23% VM kiawe charcoal did not have any effect on hydrolytic enzyme activity, in contrast to the 23% VM corncob charcoal. Therefore, VM content appears to be too broad a measurement that does always predict carbon bioavailability. Instead, we determined that a better indicator of the bioavailability of charcoal was the presence of extractable molecular compounds, as measured by the GC-MS analysis, since we detected several compounds in the 23% VM corncob charcoal but none in the kiawe. We showed that the removal of the acetone-extractable fraction from charcoal reduced its effect on microbial activity, whereas its addition to fungal inoculum increased their growth and activity.

In general, we found that high VM charcoal with a labile carbon pool can result in nitrogen immobilization. However, the effects of charcoals on soil nitrogen are less clear. While the 34% VM corncob charcoal resulted in strong nitrogen immobilization, the 23% VM corncob only reduced the rate of nitrogen mineralization. This might be related to the types of carbon compounds present and the nitrogen limitations in the soil. The 7% volatile matter corncob charcoal did not affect the rate of soil nitrogen mineralization in the first incubation. However, it reduced the rate in the second, which might be due to the observed enhancement in nitrification and thus turnover.

In our final experiment, contrary to reports in the literature, we failed to show any substantial enhancement in CEC upon the addition of charcoals to soil (2.5% weight/weight), despite measuring an increase in net negative charge for the 23% VM corncob and kiawe charcoals upon incubation. Our inability to detect changes might be due to the high potential charge of our Hawaii soils, masking the effect of charcoal, since only slight increases in the soil pH_0 were observed even at the 20% rate. Secondly, we measured CEC measured under acidic conditions, whereas previous studies have reported potential CEC at pH 7 or the summation of cations.

The total acidic surface groups were greatest in the 7% volatile matter corncob charcoal, but the phenolic and lactonic groups increased more rapidly upon oxidation for the 23% volatile matter corncob charcoal. However, we believe that the estimate of the surface groups of our charcoals is compromised by the Boehm titration method due to the presence of bio-oils and minerals. These results illustrate the limitations in this technique when analyzing charcoals. Additionally, charcoals might need pretreatment to leach these materials from the charcoal before analysis. Subsequent analyses for surface acid functional groups (e.g. Boehm titration)

and the charge fingerprint (e.g. pH_0, ZPNC, CEC, and AEC) should be performed to determine the effect or interference caused by these materials.

Our research has illustrated the many challenges involved with the use of charcoal as a soil amendment, particularly due to the complicated nature of charcoal. Charcoals represent a vast array of materials with contrasting chemical structure and composition. These differences can substantially affect soil processes, particularly those involved in soil fertility. We showed that the charcoals associated with diminished plant growth contain a bioavailable carbon source and result in enhanced microbial activity and nitrogen immobilization in short-term studies. However, we also showed that not all high VM charcoals stimulate microbial activity. As a result, our prediction of charcoal behavior based upon its VM content is limited. Furthermore, more research must be performed to further characterize the VM content in charcoals, such as with Py-GC MS and HPLC. This will help explain how differences in chemical composition affect bioavailability of charcoal, and thus improve our interpretation of the effect of VM content on soil biology. Secondly, high VM charcoals appear to oxidize and develop negative charge more quickly than low VM charcoals. However, we were unable to show that a significant enhancement in soil CEC. Further research is needed to determine the time, soil type, and charcoal quantity or pretreatment suitable for the enhancement of the soil CEC under field conditions.

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